A NOTE ON THE FATE OF TARTRATES IN THE BODY.*

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There is a notable paucity of literature relative to the influence and fate of tartaric acid introduced into the organism. In the well known purgative action of tartaric acid salts it is assumed that only small quantities of the drug are absorbed owing to the formation of insoluble calcium compounds.

Thus Wallace and Cushny[1] state that sodium tartrate is absorbed at about the same rate as the sulphates. It is, therefore, probable that only small quantities of these salts are in the circulation at any given moment. On the other hand, scattered here and there throughout the literature may be found instances of poisoning by tartaric acid and its salts, with occasional fatal results, even though only relatively small amounts of these compounds have been ingested.

Apparently the first to investigate the toxicity of tartaric acid was Chabrié,[2] who compared the poisonous action of the four stereoisomeric compounds after intraperitoneal injection. All four compounds possess toxicity, although the optically active acids are apparently the most poisonous. Brion[3] attempted to determine to what extent the different tartaric acids are eliminated in the urine when given by way of the mouth to dogs. He demonstrated that no one of the tartaric acids is completely burned or transformed in the organism, as much as 42 per cent. of the racemic form reappearing in the urine. The other varieties were eliminated in smaller quantity, but even the d-tartaric acid was found again in the urine to the extent of 25 to 30 per cent. of the amount ingested. More recently Neuberg and Saneyoshi[4] have repeated the experiments of Brion, and in the main have corroborated his results, although they lay emphasis upon the fact that the excretion of tartaric acid is quite variable. From these statements it is evident that tartaric acid given by mouth is apparently much more difficult of combustion than are most other organic acids. In the last two investigations cited no mention is made of a toxic action of the compounds studied.

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[1] Wallace, G. B., and Cushny, A. R., *Am. Jour. Physiol.*, 1898, i, 411.

[2] Chabrié, C., *Compt. rend. Acad. d. sc.*, 1893, cxvi, 1410.

[3] Brion, *Ztschr. f. physiol. Chem.*, 1898, xxv, 283.

[4] Neuberg, C., and Saneyoshi, S., *Biochem. Ztschr.*, 1911, xxxvi, 32.

So far as we are aware no observations have been recorded showing the fate of tartrates after subcutaneous injection, except those of Baer and Blum[5] whose conclusions we consider erroneous. MacCallum[6] has stated that tartrates may be administered subcutaneously with impunity, although no data of actual injections are presented.

We have carried out such an investigation. Since previous experience had demonstrated that rabbits receiving subcutaneous injections of tartrates refuse food, it became essential to make our observations during a period of inanition. The quantity of tartrate normally present in the urine during a fore-period of three days was first estimated. The following day an injection of tartrate was given and the elimination observed again for a period of three days, and finally the quantity excreted during an after-period of three days was determined. The urines voided during a period were combined and a single tartrate estimation was carried through for each period, the method of Brion, as modified by Neuberg and Saneyoshi,[7] being employed. The racemic tartaric acid was administered and in each instance was neutralized with sodium carbonate. In two cases a histological study was made of the kidneys and liver. The appended tables show the details of the observations made.

EXPERIMENT I.

Rabbit, Weighing 1,900 Grams.

Period in days.		Tartaric acid in gm., introduced subcutaneously.	Urine.		Remarks.
			Volume in c.c.	Tartaric acid in gm., calculated as tartaric acid.	
Fore-period	1	0.000	80	0.825	
	2		68		
	3		38		
Experimental period	1	0.528	12	0.000	Water-clear urine throughout.
	2	0.000	16		
	3	0.000	24		
After-period	1	0.000	84	0.013	Water-clear urine throughout. Neck twisted to one side. Prostrated. Killed.
	2		172		
	3				

[5] Baer, J., and Blum, L., *Beitr. z. chem. Phys. u. Path.*, 1907, x, 80; 1908, xi, 101; *Arch. f. exper. Path. u. Pharmakol.*, 1911, lxv, 1. Underhill, F. P., *Jour. Biol. Chem.*, 1912, xii, 115.

[6] MacCallum, J. B., *Am. Jour. Physiol.*, 1903–4, x, 101.

[7] Neuberg, C., and Saneyoshi, S., *loc. cit.*

Histological Examination. Kidney.—A large proportion of, but not quite all, the proximal convoluted tubules show complete destruction of their epithelium, which remains as a coarse granular mass entirely filling the tubule. There is very little tendency to cast formation in the convoluted tubules. The earliest stage of the process, which is seen also in the distal convoluted tubules, is a vacuolization of the epithelium. Glomerules and interstitial tissues show no changes, but there is blood in the pelvis of the kidney. Collecting tubules show little change, but the majority of the tubules in the pyramids show hyaline casts.

Liver.—Normal.

EXPERIMENT II.

Rabbit, Weighing 2,080 Grams.

Period in days.		Tartaric acid in gm., introduced subcutaneously.	Urine.		Remarks.
			Volume in c.c.	Tartaric acid in gm., calculated as tartaric acid.	
Fore-period	1	0.000	80	0.450	
	2		80		
	3		64		
Experimental period	1	0.511	20	0.023	Throughout the experimental period this animal remained in excellent condition. Water-clear urines.
	2	0.000	44		
	3	0.000	132		
After-period	1	0.000	140	0.012	Water-clear urines. No autopsy was made.
	2		154		

EXPERIMENT III.

Rabbit, Weighing 1,580 Grams.

Period in days.		Tartaric acid in gm., introduced subcutaneously.	Urine.		Remarks.
			Volume in c.c.	Tartaric acid in gm., calculated as tartaric acid.	
Fore-period	1	0.000	90	0.325	
	2		84		
	3		78		
Experimental period	1	0.780	20	0.000	Water-clear urines.
	2	0.000	32		
	3	0.000	118		
After-period	1	0.000	112	0.112	Water-clear urines. Animal very weak. No autopsy performed.
	2		112		

EXPERIMENT IV.

Rabbit.

Period in days.		Tartaric acid in gm., introduced subcutaneously.	Urine.		Remarks.
			Volume in c.c.	Tartaric acid in gm., calculated as tartaric acid.	
Fore-period	1	0.000	56	0.749	
	2		122		
	3		78		
Experimental period	1	0.765	28	0.000	Water-clear urines.
	2	0.000	10		
	3	0.000	6		
After-period	1	0.000	6	0.071	Water-clear urines. Animal in comatose condition.
	2		12		

At autopsy the kidneys were soft and hemorrhagic. All tissues were edematous. The peritoneal cavity contained 50 c.c. of a pale yellow fluid which clotted readily.

Histological Examination. Kidney.—The changes are much more extensive than in the rabbit of experiment I. Practically all the proximal convoluted tubules are devoid of living epithelium; in some there is nothing left above the basement membrane, and in others the granular debris fills the tubule. The distal tubules are relatively unaffected, but somewhat vacuolated, as are the loops of Henle and the collecting tubules, which are all entirely plugged with clots. There is a little interstitial hemorrhage and some corpuscles in the tubules, but not many. The glomerules are unchanged.

Liver.—Normal.

From the data it is apparent that only an extremely small portion at most of the injected tartrate reappeared in the urine. There are at least two possible explanations that may account for the non-appearance of the introduced salt. It may be assumed that it was oxidized within the body, or perhaps otherwise broken down into utilizable compounds.[8] In view of the results reported by Brion, and Neuberg and Saneyoshi, this hypothesis seems hardly probable. It appears much more reasonable to suppose that the deleterious influence of the tartrate upon the secretory power of the kidney is sufficient to prevent the exit of the salt by this channel. The fact that the convoluted tubules are specifically acted upon, whereas the glomerular apparatus remains practically unharmed, suggests that

[8] Baer, J., and Blum, L., *loc. cit.*

tartaric acid salts are usually eliminated by the convoluted tubules, and that in this instance at least any possible vicarious function on the part of the glomerules is prohibited. In only one experiment (experiment 4) could the objection be raised that the tubular mechanism was so plugged with debris as to prevent the possibility of the passage of a tartrate solution. Indeed, in some instances the daily volume of urine after tartrate administration was greater than on the days of the fore-period.

CONCLUSIONS.

Under the experimental conditions outlined, sodium tartrate subcutaneously introduced into rabbits fails to reappear in the urine. It is concluded that the disintegrative influence of the salt upon the convoluted tubules is sufficient to account for the failure of the salt to be eliminated.

No evidence of a vicarious function on the part of the glomerulus was observed.

TARTRATE NEPHRITIS, WITH ESPECIAL REFERENCE TO SOME OF THE CONDITIONS UNDER WHICH IT MAY BE PRODUCED.*

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PLATE 20.

The subcutaneous administration of sodium tartrate to rabbits and dogs may cause a pronounced disintegrative action upon the epithelium of the kidney. Associated with the change in the convoluted tubules there is to be noted a greatly diminished urine volume and a marked decrease in the elimination of certain of the urinary constituents.[1]

In view of the fact that the changes induced in the kidney by tartrate are confined almost exclusively to the tubular epithelium, thereby affording a means of studying various phases of renal secretion with practically intact glomerular structure, an investigation has been made of some of the conditions under which this experimental nephritis may be induced. Such a study is of especial significance when it is recalled that tartaric acids or their salts may enter rather extensively into the dietary; that purgation is frequently induced by tartrates; and that, in certain instances of chronic kidney involvement, cream of tartar water is often prescribed for the promotion of diuresis.

METHODS.

In general we have employed the rabbit as our experimental animal, although in some instances dogs have been used. The tartaric acid injected was an old, beautifully crystallized product of

* Received for publication, April 21, 1913.

[1] Underhill, F. P., *Jour. Biol. Chem.*, 1912, xii, 115.

Trommsdorf, and preparations made by Baker. The acid was of the racemic variety. The sodium salt was always injected, the free acid never being administered. The various methods followed in our work were those commonly in use and call for no especial explanation. Sections of the organs were preserved in Zenker's and Orth's fluids and in 5 per cent. formalin.

THE INFLUENCE OF DOSAGE UPON THE PRODUCTION OF TARTRATE NEPHRITIS IN FASTING PHLORHIZINIZED ANIMALS.

In a former paper[2] the general features of tartrate nephritis have been reviewed; but for the sake of completeness we have inserted here some of the experiments previously published with a different object in view. Our plan has been to study the phenomena provoked by tartrate injections, such as the changes in urine volume, changes in the elimination of characteristic urinary constituents, together with the histological picture presented. The results obtained from such an investigation may be of service in the interpretation of the conditions found in spontaneous renal involvement. It is well known that phlorhizin will provoke a significant glycosuria in an animal with intact kidney, but when the renal epithelium is impaired glycosuria either does not occur at all or else the amount of sugar appearing in the urine is markedly decreased This phlorhizin action, therefore, has been in general use for a relatively long time as a test for renal efficiency.

RABBIT C.

Female Rabbit, Weighing 2,200 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1911).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Nov. 21	85	1.026	Acid	0.95	1.88	Drank 80 c.c. water.
22	75	1.030	Acid	1.30	1.16	Drank 45 c.c. water.
23	75	1.030	Acid	1.18	0.87	Drank 50 c.c. water.
24	30	1.012	Alkaline	0.00	0.00	Subcutaneous injection of 1.75 gm. tartaric acid, neutralized with sodium carbonate, in 30 c.c. water. Drank 140 c.c. water.
25	40	1.015	Alkaline	0.07	0.00	Would not drink. Animal killed. Kidneys very pale and soft.

[2] Underhill, F. P., *loc. cit.*

Histological Examination. Kidney.—(Figure 1.) The majority of the convoluted tubules show necrosis of their epithelium. In some the necrotic cells remain and fill up the lumen as a granular mass; in others this mass has broken into fragments; in a few, vacuolated cell skeletons remain attached to the basement membrane. The early stage of the necrobiosis sometimes involves first the cytoplasm nearest the lumen, the nuclei persisting until all the cytoplasm has become thus altered. More often fragmentation and vacuolization of the cytoplasm and early loss of the nucleus is the chief change. The epithelium of the loops of Henle seems to escape injury, but these and the collecting tubules contain hyaline and granular casts. In the medulla the majority of the tubules contain granular and hyaline casts. No blood or epithelial casts are seen. The epithelium of the large collecting tubules is swollen in an almost spherical form, there being much vacuolation, especially about the nucleus. The glomerules seem entirely normal. There is no hemorrhage and no inflammatory reaction of any kind.

In the preliminary experiments the elimination of both total nitrogen and dextrose was followed before and after the administration of a single dose of sodium tartrate.

The protocols show the influence of a single dose of sodium tartrate upon the excretion of nitrogen and dextrose in a fasting phlorhizinized animal.

RABBIT D.

Female Rabbit, Weighing 2,200 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1911).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Nov. 21	125	1.024	Acid	1.12	2.75	Drank 70 c.c. water.
22	60	1.036	Acid	1.17	1.28	Drank 25 c.c. water.
23	50	1.040	Acid	0.92	1.19	Drank 45 c.c. water.
24	18	——	Alkaline	0.05	0.00	Subcutaneous injection of 2 gm. tartaric acid neutralized with sodium carbonate, in 40 c.c. water. Animal drank 125 c.c. water.
25	2 drops	——	——	—	0.00	Urine clots in jelly-like mass.
26	3 drops	——	——	—	0.00	Would not drink.
27	0	——	——	—		Animal died in convulsions.

Histological Examination. Kidney.—The condition is the same as in rabbit C, except that the necrosis of the convoluted tubules is almost universal. In the cortex nothing but collecting tubules are seen containing nucleated cells, and also what may be distal convoluted tubules with swollen and fused epithelium retaining still their nuclei, but in a pyknotic condition. There are fewer casts

in the medulla than in the case of rabbit C. The glomerules and interstitial tissues seem to be normal. The vacuolation of the epithelium of the large collecting tubules is striking, the nuclei frequently being small, compact, and distorted.

RABBIT H.

Male Rabbit, Weighing 2,500 Grams, Received Daily Subcutaneous Injections of 0.25 of a Gram of Phlorhizin.

Date (1911).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Nov. 29	100	1.024	Acid	0.56	2.30	Would not take water.
30	90	1.025	Acid	1.31	2.57	Would not take water.
Dec. 1	110	1.032	Acid	1.26	1.73	Drank 100 c.c. water.
2	5	——	Alkaline	0.018	0.015	Subcutaneous injection of 1.5 gm. tartaric acid neutralized with sodium carbonate dissolved in 30 c.c. water.
3	30	1.008	Alkaline	0.066	0.068	Drank 60 c.c. water.
4	20	——	Alkaline	—	Trace	

Histological Examination. Kidney.—A few segments of convoluted tubules have escaped necrosis, otherwise the specimen is similar to the sections previously discussed.

RABBIT I.

Female Rabbit, Weighing 2,300 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1911).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Dec. 5	100	1.030	Acid	2.88	4.03	Drank 40 c.c. water.
6	200	1.020	Acid	1.73	3.45	Drank 125 c.c. water.
7	90	1.030	Acid	1.84	1.40	Drank 50 c.c. water.
8	10	——	Alkaline	0.075	0.076	Drank 125 c.c. water. Subcutaneous injection of 2 gm. tartaric acid neutralized with sodium carbonate, dissolved in 30 c.c. water.
9	0					Animal killed. Fluid in peritoneal cavity. Abdomen edematous. Surface of kidneys hemorrhagic.

Histological Examination. Kidney.—A large proportion, but not quite all of the proximal convoluted tubules show complete destruction of their epithelium, which remains as a coarse granular mass entirely filling the tubule. There is little tendency to cast formation in the convoluted tubules. The earliest stage of the process, which is seen also in the distal convoluted tubules, is a vacuola-

tion of the epithelium, with retention of nuclei. Glomerules and interstitial tissues show no changes, but there is blood in the pelvis of the kidney. The collecting tubules show little change, but the majority of the tubules in the pyramids show hyaline casts.

Liver.—Normal.

RABBIT G.

Female Rabbit, Weighing 2,400 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1911).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Nov. 29	105	1.022	Acid	0.86	2.00	Would not take water.
30	60	1.035	Acid	1.04	1.49	Would not take water.
Dec. 1	85	1.036	Acid	1.36	0.98	Animal drank 80 c.c. water.
2	32	——	Alkaline	0.072	0.072	Subcutaneous injection of 1 gm. tartaric acid, neutralized with sodium carbonate, dissolved in 20 c.c. water. Animal drank 60 c.c. water.
3	3	0	Alkaline	0.007	Trace	Animal drank 30 c.c. water.
4	0					Animal killed.
						Tissues were not examined histologically.

RABBIT A.

Male Rabbit, Weighing 2,200 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1911).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Nov. 14	90	1.040	Acid	1.85	2.72	
15	100	1.026	Acid	1.60	1.80	
16	100	1.030	Acid	1.99	1.43	
17	30	——	Alkaline	0.13	0.00	Subcutaneous injection of 3 gm. tartaric acid, neutralized with sodium carbonate, dissolved in 40 c.c. water.
18	8	——	Alkaline	0.00	0.00	Animal lies in deep coma.
19	0	——				
20	0					Animal found dead.
						Tissues were not examined histologically.

DOG 1.

Full Grown Bitch, Weighing 9 Kilos, Received Daily Subcutaneous Injection of 1.5 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
Feb. 6	220	1.070	Acid	6.42	32.82	Animal drank 120 c.c. water.
7	415	1.074	Acid	11.70	43.33	Animal drank 250 c.c. water.
8	600	1.053	Acid	11.98	40.06	Animal drank 70 c.c. water.
9	125	1.008	Acid	0.16	00.00	8 gm. tartaric acid, neutralized with sodium carbonate, were subcutaneously injected dissolved in 50 c.c. water.
10	10	——	Acid	0.075	00.25	Animal has lost control of muscles and lies in cage. Vomits water taken. Animal killed. All organs appeared normal at autopsy.

Histological Examination. Kidney.—Beneath the capsule the tubules have a large lumen and low cytoplasm which stain deeply. The lumen usually contains more or less coarse granular detritus and frequently degenerated cells. Farther in, most of the cells of the proximal convoluted tubules are greatly swollen and vacuolated, so that they fill the lumen with a mere framework of cell outline, the nucleus of the cells usually persisting near the base; in a few tubules no nuclei can be seen. The basement membrane and the interstitial tissue are unaltered. Except for an increase in the amount of open space there are no abnormalities to be seen in the glomerules. There is no definite necrosis anywhere, and there are almost no casts, an occasional small hyaline cast being seen. In the pyramids the epithelium is slightly vacuolated, but not extensively. The collecting and the distal convoluted tubules are practically unaffected, as well as the loops of Henle.

Liver.—The cells are greatly vacuolated and swollen, so that the normal arrangement of the cells is obscured, the vacuolation being more marked in the center than in the periphery of the lobule, but all the cells are more or less affected. There is no necrosis or other change.

Adrenal.—Normal.

The data given above demonstrate the detrimental influence of subcutaneous injections of sodium tartrate upon the secretory activity of the kidney in phlorhizinized animals, and the reports of the corresponding histological study of the renal tissue show that tartrate administered under the experimental conditions has a specific action upon the convoluted tubules in both rabbits and dogs. In the earliest stage vacuolization alone is in evidence. Necrosis rapidly follows and the dead epithelium thrown off may form plugs in the tubules.

In general the urine voided after a typical tartrate injection is water-clear, almost colorless, and may contain a trace of protein or even clot on standing, depending somewhat upon the volume. In other cases microscopic examination reveals the presence of large numbers of hyaline and granular casts together with typical renal cells.

DOG 3.

Full Grown Bitch, Weighing 11 Kilos, Received Daily Subcutaneous Injection of 1.5 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.[1]	
March 5	240	1.070+	Acid	4.44	31.05	Drank 370 c.c. water.
6	240	1.070+	Acid	8.11	29.44	Drank 250 c.c. water.
7	307	1.060	Acid	8.79	29.75	Drank 290 c.c. water.
8	442	1.035	Acid	3.05	12.88	Subcutaneous injection of 10 gm. tartaric acid, neutralized with sodium carbonate dissolved in 50 c.c. fluid. After injection animal vomited repeatedly. Could not drink because of vomiting.
9	335	1.050	Acid	7.68	15.12	
10	209	1.045	Acid	1.27	04.06	
11	240	1.016	Acid	1.08	01.02	Animal in very weak condition. Killed. All organs seemed normal at autopsy.

Histological Examination. Kidney.—The section resembles that of dog 1 except that a large proportion of the tubules is affected. The subcapsular tubules are vacuolated fully as much as the others, and the collecting tubules show more vacuoles than do those in dog 1, although they are not swollen so much as the convoluted tubular epithelium. In the center of some of the convoluted tubules the remaining epithelial fragments are fused together into a dense mass, but there is little or no cast formation, there being a few granular casts in the pyramids.

Liver.—Centers of the lobules show many coarse vacuoles resembling fatty infiltration, with a few finer vacuoles towards the periphery.

The kidneys may be pale and watery, or hard with tiny hemorrhages on the surface. The first type is the more common and comparison of the weights of these kidneys with those of normal starving rabbits has shown that the pathological specimens weigh at least twice as much as the kidneys taken from normal fasting rabbits.

Inspection of the tables will show in a general way that the effect produced upon the composition of the urine is not closely related

to the amount of tartrate injected. Thus, rabbit A, weighing 2,200 grams, received three grams of tartaric acid, whereas a rabbit of 2,400 grams (rabbit G) was given only one gram of tartaric acid, and yet the influence upon the elimination of nitrogen and dextrose was practically the same in both cases. At times experiments have been carried out in which less than 0.5 of a gram of tartaric acid injected into rabbits weighing 2,000 to 2,500 grams has been sufficient to cause a practical cessation of urine flow, or at least an inhibition of the elimination of the typical nitrogenous constituents. Small doses may sometimes entirely fail to cause any noticeable effects. When small doses are effective there is apparently little difference either in degree or kind between these effects and those produced by large doses. On the other hand, the introduction of large doses of tartrates invariably causes marked reactions.

PRODUCTION OF TARTRATE NEPHRITIS DURING INANITION WITHOUT PHLORHIZIN ADMINISTRATION.

It is conceivable that in the observations previously recorded a significant rôle may be played by phlorhizin in the induction of tartrate nephritis. Phlorhizin which has an alleged specific influence upon the kidney may render the tubular epithelium hypersensitive to the action of the tartrate, or the renal tubules may suffer damage by the combined influence of the two drugs for which neither alone could be held responsible. To obviate the effect of phlorhizin some of the experiments already outlined have been repeated exactly, with the exception that phlorhizin administration has been omitted throughout.

Since creatin is an almost constant constituent of rabbit urine during a period of starvation,[3] the estimation of this substance together with the determination of creatinin and total nitrogen has been made the criteria by which renal efficiency has been measured.

Typical protocols illustrative of this point follow.

[3] Mendel, L. B., and Rose, W. C., *Jour. Biol. Chem.*, 1911–12, x, 213. Myers, V. C., and Fine, M. S., *Jour. Biol. Chem.*, 1913, xiv, 9.

RABBIT E.

Male Rabbit, Weighing 2,200 Grams. No Phlorhizin Was Given.

Date (1911).	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Creatinin in mg.	Creatin in mg.	Remarks.
Nov. 22	105	1.012	Acid	0.85	84	9	Drank 150 c.c. water.
23	65	1.022	Acid	0.81	87	34	Drank no water.
24	70	1.020	Acid	1.22	78	63	Drank 25 c.c. water.
25	30	1.015	Alkaline	0.12	Trace; too small to estimate	Trace; too small to estimate	Subcutaneous injection of 3 gm. tartaric acid, neutralized with sodium carbonate in 40 c.c. water.
26	Less than 1 c.c.	—	——	—	——	——	Drank 100 c.c. water.
27	0						Drank 60 c.c. water.
28							Found dead.

Histological Examination. Kidney.—(Figure 2.) Necrosis of the convoluted tubules is almost universal, and the necrotic epithelium is generally disintegrated. The collecting tubules show little change and there are many casts. The glomerules and interstitial tissue seem unaffected.

RABBIT F.

Female Rabbit, Weighing 2,200 Grams. No Phlorhizin Was Given.

Date (1911).	Urine.						Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Creatinin in mg.	Creatin in mg.	
Nov. 22	115	1.011	Acid	0.74	90	21	Drank 90 c.c. water.
23	70	1.020	Acid	0.97	111	39	Drank no water.
24	60	1.025	Acid	0.90	84	48	Drank 50 c.c. water.
25	32	1.015	Alkaline	0.15	Trace; too small to estimate	Trace; too small to estimate	Drank 60 c.c. water. Subcutaneous injection of 3 gm. tartaric acid, neutralized with sodium carbonate.
26	Less than 5 c.c.						Drank 25 c.c. water.
27	0						Animal in light coma. Killed. Kidneys were pale and soft.

Histological Examination. Kidney.—The conditions are about the same as for rabbit E. In the pelvis is a mass of coagulated protein, apparently from highly albuminous urine.

In other instances experiments were planned so that urine volume only was recorded, the real object of these observations being the

histological study of the renal organs. Several protocols of this nature are appended.

Rabbit 5.—A rabbit weighing 1,900 grams was placed in a cage without food; water was given. The urine volume for twenty-four hours amounted to 120 c.c. The following day the animal received a subcutaneous injection of 1 gm. of tartaric acid dissolved in 15 c.c. of water and neutralized with sodium carbonate. The volume of urine eliminated during this twenty-four hour period was 20 c.c. The rabbit was killed the next morning. Autopsy revealed the typical soft, pale kidneys and a considerable amount of fluid in the peritoneal cavity.

Histological Examination. Kidney.—Nearly all the proximal convoluted tubules show complete destruction of their epithelium, which remains as a coarse granular mass entirely filling the tubule. Glomerules and interstitial tissues show no changes.

Liver.—Normal.

Rabbit 6.—A rabbit weighing 2,500 grams was maintained under conditions similar to those noted for rabbit 5. The volume of urine voided during the succeeding twenty-four hours amounted to 200 c.c. The next day 1.5 gm. of tartaric acid neutralized with sodium carbonate were injected subcutaneously. The urine volume for this day was 86 c.c. On the following morning the animal was killed. The kidneys seemed normal on autopsy, and fluid was absent from the peritoneal cavity.

Histological Examination. Kidney.—The tissue was relatively little affected. All the epithelium of the tubules shows more or less granular degeneration and vacuolation, but there is no necrosis. A few tubules contain corpuscles and in a few tubules in the pyramids granular casts are present. The glomerules are congested and swollen. There are no interstitial changes.

Rabbit 7.—A rabbit weighing 2,000 gm. was maintained under the previously cited conditions. For the first day the urine volume amounted to 124 c.c. On the following day the animal received a subcutaneous injection of 2.5 gm. of tartaric acid neutralized with sodium carbonate. On this day 70 c.c. of urine were voided. Autopsy on the next day revealed the characteristic pale, soft kidneys. A relatively large volume of fluid was found in the peritoneal cavity and there was an area of subcutaneous edema at the site of the tartrate injection.

Histological Examination. Kidney.—This kidney shows considerable necrosis and vacuolation of the proximal convoluted tubules, with extensive cast formation.

Liver.—Normal.

The results of the experiments justify the conclusion that the disintegrative changes observed in the kidneys of phlorhizinized rabbits are due to the action of the tartrate. Although our experience with the dog under these conditions has been much more limited, the observations indicate that in this animal also the same statement holds true. Phlorhizin seems to be without significant

influence in the reaction; the same type and degree of response may be called forth by tartrate in animals that have not received phlorhizin. Tartrate exerts a specific action upon the kidney; neither the liver nor the adrenal is affected. The dose of tartrate introduced appears to bear no direct relation to the extent of damage done to the kidney epithelium.

THE INFLUENCE OF ORAL ADMINISTRATION OF TARTRATE UPON THE KIDNEY OF FASTING RABBITS.

The widespread employment of tartrates in articles of diet, and the general employment of Rochelle salts as a purgative, make of particular interest an investigation of the influence of tartrates upon kidney structure and function when the salts are given by mouth. In order to use the results of the previous experiments as a standard of comparison the present observations were made with fasting animals. The protocols follow.

Rabbit 19.—A rabbit weighing 2,640 gm. was kept without food for three days. At the end of this period 10 gm. of tartaric acid (Baker, tested purity) neutralized with sodium carbonate were introduced into the stomach through a sound. The animal died in less than twelve hours. The organs appeared normal. Anuria has been in evidence for several hours.

Histological Examination. Kidney.—The epithelium of the convoluted tubules is fairly well preserved. There is slight vacuolation in some, and in others extensive desquamation. Most of the tubules contain granular, and in some instances, cellular debris, with occasional karyorrhexis. At the junction of the cortex and medulla is a narrow zone in which there are numerous tubules with epithelium entirely necrotic, swollen into a granular mass entirely filling the lumen. No such entirely necrosed tubules are found in either the cortex or the pyramids; in a few collecting tubules are hyaline casts, and in many convoluted tubules there is considerable nuclear fragmentation. The glomerules and interstitial tissues are normal. With Sudan III, there is found to be a diffuse slight fatty change in the convoluted tubule epithelium and a very severe fatty change in all parts of the loops of Henle. No fat is present in the collecting tubules or glomerules.

Liver.—There is a slight increase in the number of round cells in the interlobular connective tissue. The liver cells are considerably vacuolated in areas in various parts of the lobules, due to fat as shown by Sudan III. The cords are slightly narrowed as in a fasting animal.

Adrenal.—Normal.

Rabbit 21.—After a three days' fast a rabbit weighing 1,840 gm. was given 10 gm. of Rochelle salts (Merck's, crystalline product) by mouth, dissolved in 50 c.c. of water. The animal died in less than ten hours. The kidneys were large and hemorrhagic.

Histological Examination. Kidney.—Nearly all the secreting epithelium of all tubules has been destroyed by a process of vacuolation, which involves first the central part of the cytoplasm and spreads outwards until the entire cell is destroyed, the nucleus persisting last of all, becoming flat and pyknotic before disappearing. All stages of the process are present, but there are few cells in the convoluted tubules that are not somewhat affected; in some, only a narrow solid necrotic zone remains just inside the basement membrane. Henle's loops are less affected. The lumina of the tubules are full of loose cellular debris and occasional desquamated cells, but there is only occasionally any formation of casts, which are mostly granular. The glomerules show an extensive accumulation of granular material in the space, with the tufts much shrunken; there is some desquamation of endothelial cells into the glomerules, but no hemorrhage. Interstitial changes are absent, but there is edema of the areolar tissue about the hilum. With Sudan III, there is no trace of fatty change.

Liver.—Normal.

Rabbit 22.—After three days of inanition a rabbit weighing 1,660 gm. was given 8 gm. of Rochelle salts (Merck's crystalline) by mouth, dissolved in 50 c.c. of water. During the next twenty-four hours diarrhea was in evidence. The urine volume remained normal, although the nitrogen output was reduced from 0.88 to 0.31 gm. The rabbit lived about thirty-six hours. Just before death kidney secretion had stopped. The kidneys were hemorrhagic and the peritoneal cavity contained a relatively large volume of fluid.

Histological Examination. Kidney.—There is severe vacuolation of all the convoluted tubule epithelium, there being no normal cells, and most are entirely necrotic. Coagulation necrosis is prominent and cast formation is extensive. Henle's loops are generally filled with casts but the epithelium is usually well preserved. The collecting tubules are nearly all filled with hyaline casts. The glomerules occasionally contain a little granular debris, but most of them are normal. With Sudan III nearly all the tubules in the cortex show great numbers of fine fat globules; there is no fat in the collecting tubules, but the loops of Henle are very fatty. Occasionally a cast is found full of fat. There is but a trace of fat found in the glomerules.

Liver.—Normal.

Adrenal.—Normal.

Rabbit 23.—Following a three days' period of fasting, a rabbit weighing 1,480 gm. was given 5 gm. of Rochelle salts (Merck's crystalline) by mouth. The animal remained in apparently normal condition for a period of three days, when it was killed. At autopsy all tissues appeared to be normal.

Histological Examination. Kidney.—Almost normal. There is a little granular precipitate in some of the tubules, and a little granular change and vacuolation of the epithelium of a few of the convoluted tubules. No fat could be found by Sudan III.

Liver.—There is some connective tissue proliferation about the bile ducts and portal vessels. No stainable fat is present.

Adrenal.—Normal.

From the foregoing protocols it is apparent that tartrates administered by mouth to fasting rabbits are capable of calling forth pathological changes in the kidney similar in general to those produced by parenteral introduction. These observations also indicate that in order for tartrates given *per os* to produce effects at all comparable to those induced by subcutaneous administration, much greater doses are necessary.

THE ACTION OF ORAL ADMINISTRATION OF TARTRATES TO PHLORHIZINIZED RABBITS DURING FASTING.

In the previous section attention has been called to the fact that tartrates given by mouth to fasting rabbits produce pathological changes in the kidneys similar in kind to those provoked by subcutaneous introduction. Little opportunity for observance of change in urinary secretion was exhibited, since most of the animals died so soon after the ingestion of the salt. When the dose was sublethal the change in nitrogen elimination was not particularly well marked.

The present portion of this paper deals with the influence of tartrates when introduced *per os* to fasting rabbits, upon the rate and extent of elimination of water, urinary nitrogen, and dextrose, the excretion of the latter being induced by daily subcutaneous injections of phlorhizin. Examples of the results obtained follow.

RABBIT 12.

Rabbit, Weighing 1,720 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
March 25	90	1.030	Alkaline	0.80	1.79	
26	64	1.030	Acid	1.20	1.16	
27	47	1.040	Acid	1.14	1.04	
28	64	——	Alkaline	0.07	0.00	10 gm. tartaric acid, neutralized with sodium carbonate, dissolved in 50 c.c. water given by stomach sound. Animal died in 18 hrs.

[4] For eighteen hours.

Histological Examination. Kidney.—Extensive destruction of the epithelium of the convoluted tubules through vacuolation of the cytoplasm. The tubules immediately beneath the capsule are least affected, while the loops of Henle, especially at the junction with the medulla, show much coagulation necrosis. The glomerules show extensive exudation into the space with collapse of the tufts, while there are practically no casts in the tubules. With Sudan III there is no demonstrable fat in the epithelium, beyond a few droplets in the loops of Henle.

Liver.—Normal.

Adrenal.—Normal.

RABBIT 17.

Rabbit, Weighing 2,120 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
April 10	86	1.018	Acid	0.72	1.71	
11	104	1.025	Acid	1.14	2.58	
12	286	1.028	Alkaline	0.99	3.38	10 gm. tartaric acid, neutralized with sodium carbonate given by stomach sound.
13	56	1.024	Alkaline	0.62	1.24	
14	175		Acid	0.16	0.30	Animal died at the end of 12 hrs.

Histological Examination. Kidney.—There are several areas of fibrosis beneath the capsule, which presents an irregular surface as if there had been some chronic inflammation. A few tubules have calcified epithelium, and a few others near the capsule show entirely necrotic epithelium which takes a faint basic stain. A few convoluted tubules and some of the Henle loops show recent necrosis of the epithelium, but these are rare. A few glomerules show fibrosed capsules. There are no casts. With Sudan III there is extensive, but not severe, fatty degeneration of the epithelium of practically all the convoluted tubules; there is very severe fatty degeneration of Henle's tubules, but the collecting tubules are practically free from fat. There is considerable fine fatty change in the interstitial tissue of the pyramids. The glomerules show no fat. This kidney is different from all the others in respect to the chronic changes present, which undoubtedly antedate our experiment.

Liver.—Much fine vacuolation of cells with fat in the center of the lobules; otherwise no changes are apparent.

Adrenal.—Normal.

[5] For twelve hours.

RABBIT 13.

Rabbit, Weighing 1,680 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
March 25	110	1.020	Alkaline	0.96	1.12	
26	80	1.035	Acid	1.44	1.79	
27	43	1.050	Acid	1.27	1.04	
28	130	1.035	Alkaline	1.04	2.24	5 gm. tartaric acid, neutralized with sodium carbonate, given by stomach sound.
29	40	1.035	Alkaline	0.90	0.40	
30	38	1.032	Alkaline	0.90	0.80	Animal killed.

Histological Examination. Kidney.—A slight degree of vacuolation of the convoluted tubular epithelium, which is quite swollen generally and obliterates the lumen in many places. No other changes are noticeable. No casts. With Sudan III there is found a heavy fatty change in all the tubules at the junction of the cortex and medulla, but no fat is present in the cells outside this zone.

Liver.—Slight vacuolation of cells in the centers of the lobules, which is shown by Sudan III to be caused by fat.

Adrenal.—Normal.

RABBIT 14.

Rabbit, Weighing 1,480 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
March 25	140	1.020	Acid	0.96	2.00	
26	50	1.032	Acid	1.14	1.63	
27	41	1.045	Acid	1.28	1.36	
28	29[6]	1.040	Alkaline	0.21	0.70	Administered by stomach tube 2.5 gm. tartaric acid, neutralized with sodium carbonate. Animal died in approximately 18 hrs. All organs seemed normal on autopsy.

Histological Examination. Kidney.—The epithelium of the convoluted tubules is much swollen and granular, obliterating the lumen of the tubules in most places. Wherever there is a lumen it contains granular detritus, and there is a little of the same in the glomerular spaces. No necrosis, casts, or interstitial changes. With Sudan III there is found to be a light diffuse staining of

[6] For eighteen hours.

very fine granules in all the convoluted tubules, but the fatty change is severe only in the loops of Henle; and every tubule, except a few large collecting tubules at the junction of the cortex and medulla, is very fatty. There is no fat in the pyramids or in the glomerules.

Liver.—Normal.

Adrenal.—Normal.

RABBIT 20.

Rabbit, Weighing 2,020 Grams, Received Daily Subcutaneous Injection of 0.25 of a Gram of Phlorhizin.

Date (1912).	Urine.					Remarks.
	Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Dextrose in gm.	
May 1	70	1.015	Alkaline	0.58	1.40	
2	58	1.030	Acid	1.00	1.12	
3	60	1.032	Acid	1.25	1.40	
4	120	1.027	Alkaline	0.56	0.80	10 gm. Rochelle salts (Merck) administered by stomach sound.
5	80	1.030	Alkaline	1.00	1.16	
6	58	1.030	Alkaline	1.24	0.54	
						Animal killed. Kidneys seemed small and shrunken.

Histological Examination. Kidney.—Hyaline casts are very numerous in the loops of Henle, and in the proximal convoluted tubules. The epithelium is much better preserved than is usual in specimens showing so many casts, but nearly all the tubules show more or less granular detritus in the tubules. Glomerules and interstitial tissue are normal. Sudan III shows fat in a few isolated segments of Henle's loops, but no fat elsewhere.

Liver.—Normal.

Adrenal.—Normal.

A survey of the data given above leads to the conclusion that the introduction of tartrates by mouth to fasting rabbits, even in relatively large doses, is not nearly so effective in inhibiting the flow of urine and the excretion of urinary constituents as the administration of much smaller doses subcutaneously. There is an indication in some of the tables of a relatively slight diminution in the elimination of the urinary constituents under discussion. This reduction is not sufficiently marked, however, to lead one to assume the existence of any pathological change in the renal epithelium. Nevertheless, a glance at the respective histological reports will furnish sufficient evidence of a disintegrative action upon the convoluted tubules. The influence had apparently reached only the initial stages, which may account for the lack of noticeable change in the excretory

powers of the kidney. The influence upon the kidney seems hardly sufficient to account for the rapid intervention of death and it is suggested that the latter may be due to some secondary effect upon other organs or tissues.

THE INFLUENCE OF TARTRATES WHEN INTRODUCED BY MOUTH AND SUBCUTANEOUSLY TO WELL FED RABBITS.

When tartrates are given by way of the mouth to well fed rabbits there is little evidence of a change in the elimination of urine, nor is the salt so toxic as when similarly introduced into animals when fasting. The following may be cited as examples illustrative of this point.

Rabbit 24.—A well fed female rabbit, weighing 2,800 gm., was taken from the stock pen and given by stomach tube 10 gm. of Rochelle salts dissolved in approximately 50 c.c. of water. From this time food was withheld. No symptoms of any sort, except a slight diarrhea, were noticeable for a period of three days. The volume of urine for the first day amounted to 72 c.c., for the second day to 36 c.c., and for the third day to 50 c.c. The specimens showed no differences that could distinguish them from normal rabbit urine. The animal was killed at the end of the third day and at autopsy all the organs and tissues seemed normal.

Histological Examination. Kidney.—There is a very little granular debris in some of the tubules, a little vacuolation in some of the epithelium cells, and a rare hyaline cast. Sudan III shows a severe fatty change involving all the tubules in the zone between the cortex and pyramids, stopping quite abruptly on either side. There is a very small amount of fat in some of the convoluted tubules (proximal) and almost none at all in the collecting tubules in the pyramids.

Liver.—There is extensive vacuolation of practically all the parenchyma cells, without necrosis or other changes. Sudan III shows this to be diffuse fatty change, somewhat more severe in the peripheries of the lobules.

Rabbit 25.—A well fed rabbit, weighing 2,600 gm., was taken from the stock pen and immediately given by stomach tube 8 gm. of Rochelle salts. Subsequent to the salt administration food was withheld. The rabbit showed no symptoms of any kind and for a period of two days voided normal urine. The volume of urine eliminated during the first day amounted to 140 c.c., while 100 c.c. were excreted on the second day. On this day the animal was killed. Autopsy showed all the organs to be normal in appearance.

Histological Examination. Kidney.—Almost normal; there is a little granular precipitate in some of the tubules, and a little granular change and vacuolation of the epithelium of a few of the convoluted tubules constitute all the changes to be observed. No fat was found with Sudan III.

Liver.—Normal.

Adrenal.—Normal.

Comparison of the effects produced by administration of tartrates *per os* to rabbits in a state of inanition and in a well fed condition shows in each instance the existence of what may be considered the initial stage in the process of renal disintegration. In the former case the pathological change appears to progress to a point considerably beyond the stage reached by the abnormal process in the well fed animal. In other words, even in the animal previously well fed, tartrates given by mouth are capable of initiating pathological changes in the kidney. Nevertheless, these abnormalities are much more in evidence when the animal has been starved previously for a short period. It is apparent, therefore, that the nutritive condition of the animal plays a part in the degree of effect produced by the introduction of tartrates by mouth.

In order to test the correctness of this hypothesis, subcutaneous injections of tartrates have been given to well fed animals. Administration of tartrates in this manner rarely fails to induce marked effects in the fasting rabbit. A procedure like that outlined above should, therefore, decisively answer the question under discussion.

The experiments below are cited as examples bearing upon this point.

Rabbit 26.—A well fed rabbit, weighing 2,000 gm., was taken from the stock pen and immediately given a subcutaneous injection of 1 gm. of Rochelle salts (0.5 gm. per kilo). The animal showed little abnormality during the three days of observation. On the first day 54 c.c. of apparently normal urine were voided; on the second day, 150 c.c. of very pale urine; and on the third day 88 c.c. of urine of the same character. The rabbit was killed at the termination of the third day, and autopsy showed all organs and tissues to be normal in appearance.

Histological Examination. Kidney.—There is considerable vacuolation of the convoluted tubule epithelium, but no necrosis. A few hyaline casts are to be found in the collecting tubules. There is a little blood in some of the collecting tubules, but no source was found for this. Other structures were normal. All the epithelial cells take a diffuse pale yellow stain with Sudan III, not resembling fat droplets in color.

Liver.—Contains much blood. There is slight vacuolation of the liver cells throughout the lobule.

Rabbit 27.—Under the conditions cited for rabbit 26, to a rabbit weighing 2,000 gm. 2 gm. (1 gm. per kilo) of Rochelle salts were introduced subcutaneously. No symptoms were noted except that the urine voided was very pale, being almost colorless. The volume for the first day was 27 c.c., for the second day 130 c.c., and for the third day 100 c.c. On the third day the animal was killed. Autopsy revealed only apparently normal organs.

Histological Examination. Kidney.—A large proportion of the (distal) convoluted tubules show complete necrosis of their epithelium which in some is still granular and in others is fused into hyaline casts. The rest of the convoluted tubules practically all contain either granular debris or hyaline casts, even when their epithelium is intact. The limbs of Henle's loops are usually distended by hyaline casts, while there is some necrosis of the epithelium of the loops themselves. The glomerules and collecting tubules are normal, while the smaller collecting tubules contain many casts. There are some patches of connective tissue proliferation in the medulla at the base of the pyramids. Sudan III shows no trace of fat.

Liver.—Normal.

Adrenal.—Normal.

From these experiments it is evident that the effects produced by tartrates subcutaneously administered to rabbits in an initial well fed condition may be as marked as those evoked during a period of inanition, although from our experience we have gained the impression that the changes induced in well fed animals by the subcutaneous introduction of tartrates are in general somewhat less pronounced than in the starving rabbit. For instance, in a well fed animal larger doses are necessary in order to evoke comparable results, both from the standpoint of urine elimination and the action upon the renal epithelium. Nevertheless, it must be concluded that the nutritive condition has apparently less relation to the subcutaneous introduction of tartrates than to the ingestion of these compounds.

THE INFLUENCE OF ALKALI ADMINISTRATION UPON THE DEVELOPMENT OF TARTRATE NEPHRITIS.

In view of the beneficial action, as alleged by Fischer,[7] exerted in nephritic conditions by alkali, it appeared of importance to determine to what extent the pathological renal changes induced by tartrates would be affected by an assured sufficiency of alkali in the body. Under normal conditions the urine of the rabbit shows an alkaline reaction. During inanition a change in the balance of acids and bases occurs as evidenced by the markedly acid character of the urine.

According to Fischer's view, an accumulation of acid in the kidney cells is responsible for the production of the nephritic condi-

[7] Fischer, M. H., Nephritis, New York, 1912.

tions in general. It is difficult to understand how a production of acid takes place in the kidney by the subcutaneous injection of a practically neutral (or slightly alkaline) solution of tartrate. An inspection of the protocols presented above will show that in general when solutions of tartrates are given hypodermically the urine assumes a strongly alkaline reaction. On the other hand, it is conceivable that the loss of alkali during inanition may play a part in tartrate nephritis, for instance, by rendering the renal epithelium unusually sensitive to substances like tartrate that are not readily combustible in the organism.

Nevertheless, in spite of these considerations, our observations with tartrates are not in harmony with Fischer's hypothesis, since tartrates, among other salts, are mentioned by him as being of value in the prevention of nephritis. We find, however, that tartrates are capable of provoking a most severe pathological change in the renal epithelium. Fischer probably assumed that tartrates are readily burned to carbonates, in a manner similar to certain other salts of organic acids.

In our experiments concerning the influence of alkali administration upon tartrate nephritis we have taken as an index of alkali sufficiency the maintenance of an alkaline (to litmus) urine. To obtain a permanently alkaline urine in the fasting rabbit two procedures have been followed. In the first place sodium carbonate was employed as the alkali, in other cases sodium acetate and sodium citrate were given, the latter course being pursued with the idea that the greater portion of these salts would only slowly burn to the form of carbonate, and hence might be less rapidly eliminated than when the carbonate was given directly. In order to insure an alkaline urine in the fasting rabbit it is necessary to administer by mouth approximately two grams daily of any of the salts mentioned above. Beyond a slight diarrhea, none of these compounds gave rise to any noticeable symptoms. In control experiments the salts were given on three successive days during inanition, and throughout this period the urine voided exhibited varying degrees of alkalinity. At the end of this time the animals were killed and the kidneys examined histologically. Typical reports concerning specimens thus procured follow.

Sodium Carbonate Administration. Rabbit 37.—There are no distinct pathological changes of any sort beyond a slight precipitate of protein in the convoluted tubules.

Sodium Acetate Administration. Rabbit 38.—There are no pathological changes.

Sodium Citrate Administration. Rabbit 39.—The epithelium of the convoluted tubules is swollen and there is some vacuolation. In the pyramids there are a few foci of acute interstitial inflammation, probably a local infection.

It is evident from these reports that the administration of alkali, such as sodium carbonate, or salts, as sodium acetate, that may form carbonate in large measure, is without significant influence in the production of pathological changes in the renal epithelium. Sodium citrate possibly behaves in an analogous manner, although in the single instance observed a slight action appeared to be exerted upon the convoluted tubules, although this may have been associated with the local infection which produced changes in the interstitial tissue.

With rabbits 40, 41, and 42, in contrast with the earlier protocols, it may be seen that little or no influence upon nitrogen elimination was manifested by the subcutaneous injection of sodium tartrate to fasting rabbits that had received alkali or salts forming alkalies. The dose (0.5 of a gram per kilo) of tartaric acid, however, was relatively small, although it must be granted that this quantity of tartaric acid is usually sufficient to provoke a significant nephritis in the fasting rabbit (rabbit G). The histological report on these kidneys agrees in general with the excretion of nitrogen.

Rabbit 40.—The epithelium of the convoluted tubules is swollen so that practically no lumen remains; this is accompanied by considerable vacuolation, but no definite necrosis and no cast formation. The glomerules and interstitial tissues are normal.

Rabbit 41.—Shows but little change.

Rabbit 42.—There is very slight swelling and vacuolation of the epithelium of the convoluted tubules.

THE INFLUENCE OF ALKALI UPON THE DEVELOPMENT OF TARTRATE NEPHRITIS. (SMALL DOSES OF TARTRATE.)

Rabbit 40; Weight 2,800 Grams.

Date (1912).	Water intake in c.c.	Urine.				Remarks.
		Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	
Dec. 5	0	40	1.030	Acid	0.86	
6	55	55	1.044	Alkaline	0.90	2 gm. sodium carbonate by mouth.
7	25	70	1.060	Alkaline	0.83	0.5 gm. tartaric acid per kilo, neutralized with sodium hydroxide, subcutaneously injected.
8	75	45	1.030	Alkaline	0.88	
9	10	25	1.038	Alkaline	0.71	

Rabbit 41; Weight 2,140 Grams.

Dec. 5	50	35	1.030	Acid	0.52	
6	50	85	1.040	Alkaline	1.57	2 gm. sodium carbonate by mouth.
7	50	95	1.050	Alkaline	1.66	0.5 gm. tartaric acid per kilo, neutralized with sodium hydroxide, subcutaneously injected.
8	35	53	1.040	Acid	1.65	
9	50	50	1.050	Acid	1.64	

Rabbit 42; Weight 1,720 Grams.

Dec. 5	0	30	1.040	Acid	0.70	
6	50	40	1.030	Alkaline	0.81	2 gm. sodium citrate by mouth.
7	50	95	1.034	Alkaline	0.89	0.5 gm. tartaric acid per kilo, neutralized with sodium hydroxide, subcutaneously injected.
8	35	43	1.040	Alkaline	0.93	
9	25	35	1.030	Acid	0.84	

It has been shown above that doses of tartaric acid approximating one gram per kilo of body weight given subcutaneously to fasting rabbits invariably produce significant changes both in the elimination of urinary constituents (rabbits H and I), and in the renal epithelium (rabbits C and D).

The tables illustrating the experiments carried out with rabbits 43 and 44 present the results obtained when tartaric acid in doses of one gram per kilo was injected into fasting animals receiving alkali administration similar to those previously discussed. Rabbit 45 shows the influence of the same dosage of tartrate but without treatment with alkali.

Comparison of these figures shows plainly that with the amounts given tartrates are still capable of diminishing the elimination of urinary nitrogen appreciably, even though the urine voided is constantly alkaline. The reduction of urinary nitrogen is less marked under these conditions than it is when no alkali has been administered.

Rabbit 43.—There is considerable precipitate in the convoluted tubules. Some of the epithelial cells here show a slight fusion of the cytoplasm, and in Henle's loops there is considerable necrosis with little cast formation.

Rabbit 44.—There is severe necrosis involving all of the distal and most of the proximal tubules; the same thing occurs in the loops of Henle. Many granular and hyaline casts are present. The glomerules and interstitial tissue are normal.

Rabbit 45.—Most of the distal convoluted tubules have lost their epithelium, not by coagulation necrosis, but apparently by a lysis, which leaves a skeleton of many of the cells. There is practically no coagulation necrosis, except in a few loops of Henle where occasional casts are also found. The glomerules are unchanged.

THE INFLUENCE OF ALKALI UPON THE DEVELOPMENT OF TARTRATE NEPHRITIS. (LARGE DOSES OF TARTRATE.)

Rabbit 43; Weight 1,200 Grams.

Date (1912).	Water intake in c.c.	Urine.				Remarks.
		Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	
Dec. 10	0	59	1.030	Acid	0.87	
11	25	40	1.030	Alkaline	0.96	1.5 gm. sodium carbonate by mouth.
12	125	100	1.030	Alkaline	0.60	2 gm. sodium carbonate by mouth. 1 gm. tartaric acid per kilo, neutralized with sodium carbonate, subcutaneously injected. 2 gm.
13	30	35	1.030	Alkaline	0.25	sodium carbonate by mouth.

Rabbit 44; Weight 1,200 Grams.

Dec. 10	0	45	1.024	Acid	0.56	
11	25	50	1.036	Neutral	0.70	1.5 gm. sodium citrate by mouth.
12	75	44	1.016	Alkaline	0.20	2 gm. sodium citrate by mouth. 1 gm. tartaric acid per kilo, neutralized with sodium carbonate, subcutaneously injected. 2 gm.
13	25	155	1.011	Alkaline	0.45	sodium citrate by mouth.

Rabbit 45; Weight 1,200 Grams.

Dec. 10	0	44	1.014	Acid	0.31	
11	35	55	1.020	Acid	0.88	
12	100	18	——	Slightly acid	0.11	1 gm. tartaric acid per kilo, neutralized with sodium carbonate, subcutaneously injected.
13	0	50	——	Slightly acid	0.14	

Although the number of experiments carried through is too small to permit a final conclusion to be drawn, it is indicated that the administration of alkali may somewhat diminish certain of the effects usually produced by the subcutaneous introduction of tartrates into fasting rabbits. Stated specifically, alkali treatment under the conditions outlined allows a greater elimination of urinary nitrogen than is obtained in animals subjected to analogous conditions, with the exception that alkali was withheld. From a study of the tissues there is evidence that the alkali had a slight beneficial influence upon the pathological manifestation in the renal tissues themselves.

SUMMARY.

Salts of tartaric acid administered subcutaneously to fasting phlorhizinized animals exert a markedly detrimental influence upon the secretory efficiency of the kidney, which is indicated by a greatly lessened output of certain typical urinary constituents. A histological study of the nephritic kidney demonstrates that the salts act specifically upon the epithelium of the convoluted tubules, and to a less extent upon the tubules of the loops of Henle, the glomerules, and interstitial tissue remaining unharmed. In the disintegrative process taking place, vacuolation first occurs, is rapidly followed by necrosis, and finally the dead cells or their debris may entirely fill the lumina of the tubules and form granular and hyaline casts.

There is no strict relation between the dose of tartrate and the extent of damage inflicted. While large doses invariably induce a well marked response small doses may at times produce effects equally significant.

Tartrates introduced into fasting animals call forth symptoms practically identical with those observed in fasting phlorhinized

animals. It is therefore apparent that in the establishment of the pathological condition under discussion phlorhizin is without significant influence.

Neither the liver nor the adrenal exhibits any detrimental effect from the injection of tartrates.

The introduction of tartrates by way of the mouth to fasting rabbits is not nearly so effective in the production of nephritic symptoms as the administration of much smaller doses subcutaneously. In general, under the former circumstances the initial stages only of epithelial disintegration of the convoluted tubules obtain, which, however, are scarcely sufficient to account for the rapidity with which death usually ensues.

Although in well fed animals distinct pathological changes in the kidney are induced by the introduction of tartrates *per os,* these abnormalities are less in degree, but similar in kind, than those provoked under like conditions in the fasting animal. When tartrates are given subcutaneously to well fed rabbits the effects evoked are somewhat less pronounced than when the salts are injected into fasting animals. From these facts it is evident that the state of nutrition plays a part in the development of tartrate nephritis.

It is indicated that the introduction of a sufficiency of alkali to animals in a state of fasting permits a greater elimination of urinary constituents during tartrate nephritis than obtains under similar circumstances when the alkali is omitted. Histologically there is evidence that the administration of alkali exerts a slight modifying action.

EXPLANATION OF PLATE 20.

FIG. 1. Rabbit C. Section of kidney of rabbit receiving phlorhizin and sodium tartrate. Necrosis of convoluted tubules. The glomerules are normal.

FIG. 2. Rabbit E. Section of kidney of rabbit receiving sodium tartrate only. Necrosis of convoluted tubules. The glomerules are normal.

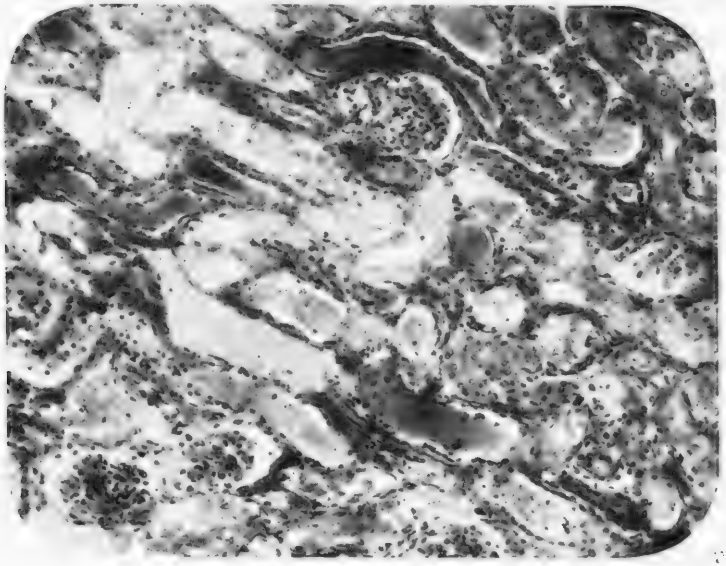

FIG. 1.

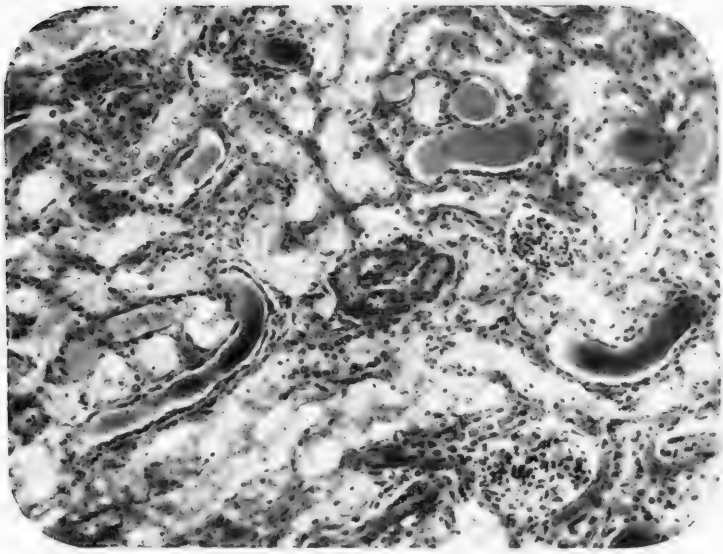

FIG. 2.

(Underhill, Wells, and Goldschmidt: Tartrate Nephritis.)

A STUDY OF RENAL SECRETION DURING TARTRATE NEPHRITIS.*

By FRANK P. UNDERHILL, H. GIDEON WELLS, and SAMUEL GOLDSCHMIDT.

(From the Sheffield Laboratory of Physiological Chemistry of Yale University, New Haven, and the Pathological Laboratory of the University of Chicago, Chicago.)

Plate 21.

Our knowledge of normal kidney function has been gained in large measure from a study of the elimination of both normal and abnormal urinary constituents in experimentally produced nephritis. Experimental nephritides have also been responsible for many of the present conceptions concerning spontaneous nephritis in man.

The pathological states hitherto subjected to observation have been evoked usually by the injection of one of a short list of poisons, as cantharidin, potassium dichromate, uranium nitrate, sodium arsenate, etc. A survey of the literature will demonstrate, however, that none of these poisons acts specifically upon either the vascular or tubular mechanism of the kidney. Both parts are more or less involved, although one may be affected much more than the other. To quote from one of the recent additions[1] to the literature of experimental nephritis, "Cantharidin, potassium dichromate, uranium nitrate, and sodium arsenate produce in the dog an acute nephritis in which both the vascular and the epithelial elements of the kidney are involved." From a perusal of the literature of the subject under discussion one gains the impression that the vascular mechanism is much more easily thrown out of adjustment than is the epithelial apparatus. Expressed differently, it is probable that in most instances of experimental nephritis the tubular epithelium is usually involved at a later period than the glomeruli and that when a tubular nephritis has been induced the glomerule is also injured to a greater or less extent.

* Received for publication, April 21, 1913.

[1] MacNider, W. de B., *Jour. Med. Research*, 1912, xxi, 79.

In other communications[2] we have called attention to the observation that tartrates, subcutaneously introduced, especially to fasting rabbits, produce an almost specific action upon the convoluted tubules, leaving the vascular mechanism practically unharmed. An animal treated in this way presents an excellent opportunity for a study of the secretory power of the kidney in well marked tubular nephritis, and the results obtained should, by a process of exclusion, yield at least a partial interpretation of the normal mechanism of renal secretion.

The investigation which is here presented was planned to determine the mode of elimination of a mixture of sodium chloride and urea dissolved in water, introduced intravenously into fasting rabbits with tubular nephritis induced by tartrates. It is obvious that by the method adopted different degrees of nephritis would be produced and that the extent of renal secretion should be a measure of the damage done to the kidney. To demonstrate further the correlation between kidney injury and secretory power, sections of the organs under discussion were studied histologically. Furthermore, since the experimental condition of fasting might play a part, we have determined in some of the experiments the secretory power of the kidney of the fasting rabbit without tartrate injection.

METHODS.

In the observations here recorded a crystalline preparation of the racemic form of tartaric acid was used for the production of nephritis in rabbits. The acid was subcutaneously introduced in the form of the practically neutral sodium salt. Kahlbaum's preparations of sodium chloride (*pro analyse*) and urea were dissolved in distilled water, the sodium chloride being present to the extent of 0.9 per cent., and enough urea being added to make an approximately 2.3 per cent. solution. Of this mixed solution fifty cubic centimeters were slowly injected into the marginal ear vein and the chloride and total nitrogen output of the urine were estimated. Urine was obtained in twenty-four hour periods by emptying the bladder by pressure on the abdominal wall. The kidneys were preserved in Zenker's and Orth's solutions and in 5 per cent. formalin.

[2] Underhill, F. P., *Jour. Biol. Chem.*, 1912, xii, 115. Underhill, F. P., Wells, H. G., and Goldschmidt, S., *Jour. Exper. Med.*, 1913, xviii, 317, 322.

In the tables presented below are given examples of the types of excretory response to intravenously injected sodium chloride and urea, first during a period of fasting alone, and secondly, after the induction of varying degrees of injury to the renal epithelium by the subcutaneous administration of sodium tartrate.

From an inspection of the data in table I it is evident that urea introduced into the blood of the fasting animal is promptly eliminated. On the other hand, sodium chloride may be excreted in the urine to the extent of only one quarter of the quantity injected, the remainder being stored in the body. These statements hold true for other similar trials of which the experiment presented may be regarded as typical.

TABLE I.

Rabbit 28, Weighing 2,100 Grams, Was Given Water Without Food.

Date (1912).	Water intake in c.c.	Urine.					Remarks.
		Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Chloride as sodium chloride in mg.	
July 22	70	55	1.025	Alkaline	0.60	40	
23	0	40	1.020	Acid	0.50	20	
24	50	60	1.010	Acid	0.54	Trace	
25	85	80	1.015	Acid	1.02	120	Injection in ear vein of 50 c.c. sodium chloride-urea solution in 20 min. Injected 450 mg. sodium chloride. Injected 0.531 gm. nitrogen. No bad symptoms.
26	10	48	1.015	Acid	0.50	40	At the end of July 26 animal was placed in pen and well fed.
30	20	30	1.034	Acid	0.49	30	Placed in cage again without food.
31	50	46	1.020	Acid	0.61	Trace	
Aug. 1	45	30	1.022	Acid	0.18	80	Subcutaneous injection of 0.5 gm. tartaric acid neutralized with sodium carbonate, in 15 c.c. water. Urine contains protein.
2	115	196	1.012	Acid	1.24	640	Injection of same amount of sodium chloride-urea solution as on July 25. Urine contains protein and many casts. Urine light colored.
3	60	30	1.030	Acid	0.55	22	Normal colored urine.

Histological Examination. Kidney.—There are many granular and hyaline casts in the cortex, and a few in Henle's loops, but only a moderate amount of vascular degeneration of the epithelium is present. There is no distinct necrosis. There are no changes in the glomerules. With Sudan III there is a faint yellowish staining of the vacuolated cells, which are mostly in the deeper part of the cortex; distinct fat granules are found in only a few of the loops of Henle.

In the second part of the experiment it would appear that the subcutaneous injection of sodium tartrate leads to an unduly increased output of sodium chloride. This result was not obtained in all the observations. That sodium tartrate did exert an influence upon the kidney structures may be concluded from the marked reduction of the nitrogen output. This diminution cannot be ascribed to lessened urine volume. Despite the evidence of an effect upon the kidney, after the second injection of the sodium chloride-urea solution there is very little indication of any abnormality in the elimination of these compounds. This may be explained, however, by a glance at the histological report of the kidneys under discussion. It is there apparent that the tubular epithelium was damaged to a limited extent only, hence the practically normal excretion of the substances introduced.

This behavior of the fasting animal after intravenous injection of the sodium chloride-urea solution (table I) finds duplication in the data shown in table II. However, when one inspects the results obtained under similar conditions with the animal in a state of typical tartrate nephritis, an entirely different picture is presented. It is evident that the administration of the tartrate rendered the kidney almost impervious to nitrogenous compounds, but not to chlorides. The small volume of urine excreted may account for this in a measure, although it is quite apparent that the ratio of chloride to nitrogen is very much greater than that found in normal conditions. After the introduction of the sodium chloride-urea solution, the volume of urine secreted was not abnormally small. Although a large proportion of the sodium chloride injected reappeared on this day and was more than accounted for on the following day, only an exceedingly small amount of the introduced nitrogen could be demonstrated in the urine. Even on the next day the nitrogen output was not more than one half that previously given

out before the tartrate administration, although the urine volume had regained the level seen during starvation alone.

TABLE II.

Rabbit 31, Weighing 2,200 Grams, Was Given Water Without Food.

Date (1912).	Water intake in c.c.	Urine.					Remarks.
		Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Chloride as sodium chloride in mg.	
July 22	40	60	1.025	Alkaline	0.71	20	
23	85	73	1.010	Acid	0.59	20	
24	100	80	1.010	Acid	0.68	Trace	
25	100	125	1.021	Acid	1.19	210	Injection into ear vein of 50 c.c. sodium chloride-urea solution in 20 min. Injected 450 mg. sodium chloride. Injected 0.531 gm. nitrogen. No bad symptoms.
26	95	90	1.008	Acid	0.67	40	At end of this day the animal was placed in the pen and well fed.
30	20	60	1.024	Acid	0.63	60	Placed in cage again without food.
31	80	70	1.028	Acid	0.65	40	
Aug. 1	75	8	1.001(?)	Acid	0.01	20	Subcutaneous injection of 0.5 gm. tartaric acid, neutralized with sodium carbonate in 15 c.c. water. Urine contains protein.
2	20	30	1.006	Acid	0.09	380	Injection of same amount of sodium chloride-urea solution as on July 26. Urine contains protein and casts.
3	0	64	1.020	Alkaline	0.29	210	

Histological Examination. Kidney.—There is severe necrosis involving every convoluted tubule, with practically complete necrosis of all the epithelium of the tubules. The lumina of all are entirely occluded by swollen necrotic epithelium and casts, or the intermediate steps in cast formation. The glomerules, interstitial tissue, and collecting tubules seem normal. With Sudan III there is considerable diffuse orange yellow staining of the degenerated cytoplasm, with occasional well defined fat granules.

The interpretation of these results becomes clear when the histological report on these kidneys is reviewed. According to this, the only structure in the kidney that has been seriously damaged is the epithelium of the convoluted tubule, which is entirely ne-

crosed (figure 1). The small volume of urine on the day of the tartrate injection may be explained by the difficulty that a solution would experience in passing through the lumina of tubules entirely occluded with swollen necrotic epithelium and casts. From the data presented in table II, taken in conjunction with results obtained from the histological examination of the kidneys, one is justified in the conclusion that normally sodium chloride and water are eliminated through the glomerules and that the convoluted tubular epithelium is concerned in the excretion of urea. These experiments therefore confirm the older observations which have led to the conception of a dual mechanism in urine secretion. The data here presented afford no support for the theory that the two structures reciprocally perform vicarious functions; at least it is demonstrated fairly clearly that the vascular mechanism cannot take over the function of the tubular epithelium.

A somewhat less perfect exemplification of the ideas recorded above may be seen in table III, although the general result obtained points decidedly in the same direction.

TABLE III.

Rabbit 47, Weighing 1,840 Grams, Was Given Water Without Food.

Date (1912).	Water intake in c.c.	Urine.					Remarks.
		Volume in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen in gm.	Chloride as sodium chloride in mg.	
Dec. 14	0	80	1.030	Acid	0.34	65	
15	25	50	1.040	Acid	0.91	50	Subcutaneous injection of 2 gm. tartaric acid neutralized with sodium carbonate.
16	70	Few drops		Acid			Injection into ear vein of 50 c.c. sodium chloride-urea solution. Injected 450 mg. sodium chloride. Injected 0.531 gm. nitrogen.
17	0	60	1.010	Acid	0.23	240	Diarrhea.
18	125	90	1.010	Acid	0.57	225	Diarrhea.

Histological Examination. Kidney.—Most of the distal convoluted tubules have lost their epithelium. The epithelium which still persists in the convoluted tubules is low and atrophied, with small deeply staining nuclei. There is still considerable necrotic epithelial material, most of which is hyaline. A few glomerules contain some precipitated albumin.

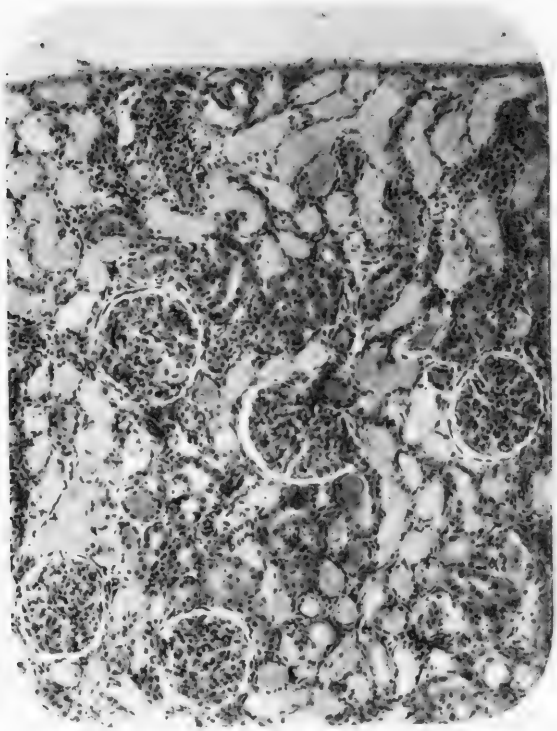

FIG. 1.

(Underhill, Wells, and Goldschmidt: Tartrate Nephritis.)

SUMMARY.

After the intravenous introduction of a solution containing sodium chloride and urea into the rabbit during pronounced tartrate nephritis, all the chloride reappears in the urine within forty-eight hours. On the other hand, the nitrogen of the urine remains far below that usually eliminated by the normal animal under the experimental conditions; in other words, little or none of the urea injected is excreted by the kidney.

In the light of the histological findings these results are interpreted to mean that under normal conditions chlorides and water are passed through the glomerular mechanism, whereas urea becomes a urinary constituent by way of the convoluted tubules. These results constitute a direct confirmation of the older observations of others concerning the elimination by the kidney of the substances under discussion.

No evidence was obtained that the glomerulus may take over the function of the tubular epithelium.

EXPLANATION OF PLATE 21.

FIG. 1. Rabbit 31. Section of kidney. Magnification × 110. Necrosis involving every convoluted tubule. The glomerules are normal.

STUDIES ON THE CIRCULATION IN MAN.

VII. The Blood Flow in the Feet.*

By G. N. STEWART, M.D.

(*From the H. K. Cushing Laboratory of Experimental Medicine of Western Reserve University, Cleveland.*)

Plates 22 to 24.

The calorimetric method already described for the measurement of the flow in the hands[1] was modified to suit the feet. This made it possible to make observations on persons who were too ill to sit in a chair for hand flow measurements.

Since the heat given off by the foot of a normal adult is considerably less than that given off per 100 cubic centimeters of tissue per minute by the hand,[2] it was necessary to construct the interior vessel so that as small an amount of water as possible would suffice to immerse the foot completely.

TECHNIQUE.

In its final form the foot calorimeter consists of an interior oval vessel of tinned copper (figure 1) packed in broken cork in an outer vessel of galvanized iron. Both vessels are somewhat wider at the anterior (toe) end than at the posterior end. The top of the inner vessel has in it an oval opening somewhat wider at the anterior end. This opening, as is obvious from the shape of the foot, must be much larger than in the case of the hand calorimeter, and when the foot has been inserted it is partly closed by the removable copper lid shown at the right lower corner of the figure. The hole in this lid allows it to be lifted easily by the fingers. On the lid, once the foot is in position, lies the thick felt collar shown at the left of the figure. Inside the calorimeter the foot rests on thick copper wire netting, shown leaning against the front of the calorimeter. This allows the water to pass freely under the foot when it is stirred by the two goose feathers, the ends of which are seen protruding from the two openings one on each side of the thermometer. The calorimeter in the figure is tilted so as to display the posterior end of the footrest lying in position on

* Received for publication, April 30, 1913.

[1] Stewart, G. N., *Heart*, 1911, iii, 33.

[2] Stewart, G. N., *loc. cit.*, p. 55.

the bottom of the inner vessel. The anterior part of the top of the inner vessel slants down somewhat towards the front in order to reduce the capacity of the vessel without encroaching on the space needed for the toes. The thermometer is protected against contact with the patient's toes by a cylinder of copper perforated with numerous holes, which passes down through the top of the inner vessel to the level of the lower end of the bulb. The orifice for the introduction of the foot is surrounded by a flange on which the lid and the felt collar rest. Vertical rings of copper surround all the orifices so as to allow the top of the inner vessel to be covered, first with a layer of broken cork, and above that with sheet cork. The upper surface of the sheet cork is varnished. The thickness of the layer of cork over the greater part of the inner vessel is 25 mm., but more in front.

The outer vessel is 260 mm. deep, 225 mm. wide in the middle, and 400 mm. long. The inner vessel is 125 mm. deep below the flange at the posterior (heel end), 135 mm. deep below the flange in the middle, 120 mm. wide in the middle, and 320 mm. long. The length of the orifice at the flange from front to back is 190 mm., the width at the middle 100 mm. The flange is about 8 mm. wide, but a little broader posteriorly. The length of the orifice from front to back when the movable lid is in position is 90 mm., and its breadth 82 mm. The height of the footrest at the heel end is 24 mm., and at the toe end 10 mm.

Figure 2 shows the position of the patient with his feet in the calorimeters when he is well enough to sit in the high chair described in the paper on measurement of the flow in the hand.[3] For the convenience of the observer the chair and the calorimeters are placed upon a platform so as to elevate the thermometers to a sufficient height above the floor to enable them to be read conveniently. By a simple method of fixation all possibility of slipping of the chair is prevented.

In the first experiments a horizontal pencil mark was drawn around the ankle, always bearing the same relation to the external malleolus, and above this a parallel line was drawn at a distance equal to the thickness of the felt collar plus the thickness of the flange. The height of the calorimeters was then adjusted so that the upper line just appeared above the felt collar, the patient sitting in the chair with his legs hanging down. The footrest inside the calorimeter was not used with this arrangement. It was found to be somewhat troublesome for the patient to maintain the same level throughout the experiment in this way. It was therefore decided to abandon it and to cause the patient to set his foot on the footrest. Before withdrawal of the foot a pencil line is now traced around the ankle just below the flange, or it may be traced above the collar, and after withdrawal of the foot a parallel line traced at a distance below the first line equal to the combined thickness of collar and flange. The object of the first procedure was to insure that in all experiments the foot should be inserted into the calorimeter to the same anatomical level, as was always done with the hands. In the case of the hands undoubtedly a fairer comparison of the flow in different individuals is obtained in this way than if the fingers were made in every case to touch the bottom of the calorimeter, since the inclusion of different proportions of the wrist and forearm would to some extent vitiate the results. From the shape of the foot, however, it is clear that the second pro-

[3] Stewart, G. N., *loc. cit.*, p. 33.

cedure leads to a much smaller variation in the portion of the foot immersed than would be the case for the hand, since in the adult the variation in the vertical distance from the sole to a given anatomical point on the ankle is much less than the variation of the length of the hand,—from the tip of the middle finger to a fixed anatomical point on the wrist, for example. As a matter of fact, in the adult patients examined the variation in the distance between the flange and a fixed point on the external malleolus when the sole rested on the footrest was small. Any error in comparing the feet of different persons was further reduced by the fact that the difference of level affected the portion of the foot (the ankle) whose horizontal cross section was smallest. For very small feet, as those of children, an additional footrest which increased the space between the sole of the foot and the bottom of the calorimeter was employed. A great advantage of the footrest, in addition to the increased comfort of the patient, is the certainty that without any attention the foot remains at the same level within the calorimeter throughout the experiment. For particular purposes in the case of children or of adults with exceptionally small feet, it may sometimes be desirable, where accurate comparison is required with other cases, to revert to the first method, although of course for comparison of the flow in the feet of one and the same person at different times or under different conditions, this would offer no advantage.

The volume of the foot was measured by displacement in the same way as that of the hand. The oval copper vessel at the left of figure 2 was made for this purpose. The cross section of the vessel must be as small as possible in order to permit as large a change of level as possible in the attached burette. Where very large or very small feet are being investigated it is of advantage to have vessels of correspondingly different size.

When patients are not well enough to sit up in the chair the flow in the feet can be measured in the way shown in figure 3. The subject is lying on his back on a movable bed with his legs hanging over the foot of the bed. The height of the calorimeters is adjusted so that the soles of the feet rest on the footrest. No complaint of discomfort was made by any of the fever patients investigated in this way.[4]

The technique of the preparatory bath is the same as that for the hands, except that instead of the patient putting his feet into the bath, the bath is generally slipped up over his feet. This is necessarily the case where patients are examined in bed. On account of the greater thickness of the feet, the period of immersion in the bath can advantageously be made longer than is necessary for the hands. For the same reason it is probable that the calculated results differ from the actual flow by a greater amount in the case of the feet than in the case of the hands. In both, of course, the observed flow establishes a minimum below which the actual flow cannot lie. It was pointed out in the previous communication that this follows from the principle of the method.

Another point of considerable importance is that the feet and legs, being less accustomed to exposure than the hands and arms, at least in temperate climates, greater precautions have to be taken to prevent vasoconstriction due to the exposure, especially of wet skin, to the air of the room. The legs are kept

[4] Stewart, G. N., *Jour. Exper. Med.*, 1913, xviii, 372.

covered as far as possible down to the bath, and afterwards down to the calorimeters. A somewhat higher bath temperature than that commonly employed for the hands is probably also advantageous for the foot measurements.

In general, measurements of the hand flow are to be preferred for obtaining information as to the condition of the circulation as a whole. It is in cases where the local conditions made it desirable to examine the foot flow, or where the patients were too ill to sit up for the hand examination, that foot flow measurements were undertaken. For the sake of comparison the hand flow was also measured wherever possible. It will be pointed out later that the ratio of the foot flow to the hand flow sometimes reveals interesting features.

The quantity of water put into each calorimeter where the feet are of average size is 2,775 c.c. In the protocols, if no quantity is mentioned, it is always to be understood that this is the amount. In the earlier observations where the capacity of the calorimeters was greater, larger quantities were employed. These are always stated in the protocols. It was afterwards decided to reduce the size of the inner vessels by cutting a segment off the bottom, so that a greater rise of the thermometers might be obtained. This is mentioned to prevent confusion in comparing the results in the earlier and later experiments. The foot volumes are larger in some of the earlier observations than in the later ones because the feet were inserted into the calorimeter to a greater depth. This, however, is not necessarily the case in the experiments in which the feet hung down in the calorimeter, but it is always the case in the experiments made where feet rested on the footrest in the calorimeters before they were reduced in size.

Water Equivalent of the Foot.—A foot was obtained from the body of a well nourished woman, 45 years old, who died of lobar pneumonia. The foot was normal in all respects and was in excellent condition. A tube of skin having been reflected, the leg bones were sawed through at the level which in an average adult foot would be just inside the flange when in position in the calorimeter. The skin was closely stitched around the bone and the line of sutures well covered with melted paraffin, to render the preparation water tight. The foot was slung by means of a thin copper wire in a bath, the temperature of which was kept constant by a regulator. The water was continuously stirred by a mechanical arrangement. The foot was put into the bath at 11.30 A.M. At 12.31 P.M. 3,275 c.c. of water were put into one of the foot calorimeters. At 1.02 P.M. the foot was quickly transferred from the calorimeter after being rapidly wiped with a towel taken out of a thermostat at 38° C., and the rise of temperature in the calorimeter was followed as in the corresponding experiment on the hand.[5]

Time.	Temperature of			Time.	Temperature of	
	Calorimeter.	Air.	Bath.		Calorimeter.	Air.
12.23			37.96°	1.25	18.30°	19.9°
12.28			37.96°	1.27	18.36°	19.8°
12.39	15.69°	18.8°	37.96°	1.29	18.39°	19.8°
12.48	15.80°	18.8°	37.96°	1.31	18.445°	19.7°
12.54	15.85°	18.8°	37.97°	1.34	18.50°	19.7°
1.00	15.895°	18.8°	37.97°	1.37	18.56°	19.7°

[5] Stewart, G. N., *Heart, loc. cit.*, p. 42.

Time.	Temperature of			Time.	Temperature of	
	Calorimeter.	Air.	Bath.		Calorimeter.	Air.
1.05	17.08°	18.95°		1.41	18.61°	19.6°
1.06	17.22°			1.45	18.665°	19.4°
1.07	17.36°	19.0°		1.50	18.71°	19.5°
1.08	17.46°			1.55	18.755°	19.6°
1.09	17.55°			2.07	18.82°	19.7°
1.11	17.69°	18.95°		2.09	18.83°	19.7°
1.13	17.825°	19.1°		2.21	18.88°	19.5°
1.15	17.92°	19.7°		2.33	18.91°	19.5°
1.17	18.02°	19.7°		2.46	18.935°	19.6°
1.19	18.10°	19.7°		2.56	18.955°	19.5°
1.21	18.175°	19.8°		3.20	18.99°	
1.23	18.235°	19.9°				

Weight of foot, 760 gm. Volume of foot, 717 c.c. The specific gravity of the foot was therefore 1.06. This is less than that of the hand, as determined before, probably because of a considerable amount of fat. After allowance is made for the small amount of heat gained by the calorimeter during the experiment, the specific heat works out at 0.71. The factor by which it is necessary to multiply the volume of a foot in order to obtain its water equivalent is accordingly 1.06×0.71, or 0.75.

The temperature of the arterial blood entering the foot was not determined, as it is certain that it cannot differ much from that of the arterial blood at the wrist, which was previously shown in a normal man to be 0.5° C. below the rectal temperature. To allow, however, for the longer path, it was assumed that at the ankle the arterial blood is 0.1° lower than at the wrist. This makes no material difference in any case, but it helps to offset the greater error in the case of the foot involved in the assumption that the venous blood leaving the foot is on the average at the temperature of the calorimeter. From the dimensions of the foot, it is probable that the difference between the temperature of the venous blood at the ankle and that of the calorimeter is greater than the difference between the average temperature of the venous blood at the wrist and that of the calorimeter. Yet in the foot, as in the hand, most of the blood must in some part of its path follow a relatively superficial course.

One point in connection with the calculations, which has, perhaps, not been made clear in the previous paper on the method, although it is sufficiently obvious, may be referred to here, lest a reader in recalculating experiments should be puzzled by small discrepancies. In allowing for the cooling of the calorimeters, the rate of cooling observed at the end of the experiment has been corrected for each period into which the observations are divided, by taking account of the difference of the average temperature of the calorimeter for the given period and the room temperature. Where the total change of temperature of the calorimeters in the whole experiment is small, this correction can be omitted without material error. In no case is the error that is introduced by omitting the correction a great one.

As no results on the feet have hitherto been published, it is necessary, in order to afford a standard of comparison for the patho-

logical cases, to cite some of the results on normal persons. These were obtained on M. C., a man aged 24 years, height 5 feet, 10 inches, weight 165 pounds, on whose hand flow numerous observations had been made; J. R., a man aged 20 years, height 5 feet, 4 inches, weight 125 pounds; C. B., a man aged 24 years, height 5 feet, 8½ inches, weight 130 pounds; and N. R., a boy aged 8 years, 9 months, height 3 feet, 11 inches, weight 54 pounds. The ages are given as at the time of writing of the present paper.

M. C.—May 18, 1911. Right foot put into bath at 2.37 P.M., and into calorimeter at 2.47½. 3,700 c.c. of water in calorimeter. Pulse (sitting) 76. The subject was sitting throughout the experiment, his foot hanging in the calorimeter.

Time.	Temperature of right calorimeter.	Notes.	Time.	Temperature of right calorimeter.	Notes.
2.47	31.17°		2.55	31.42°	
2.49	31.21°		2.56	31.45°	
2.50	31.25°	Room temperature 26.4° C.	2.57	31.48°	
			2.58	31.51°	Room temperature 26.4° C.
2.51	31.27°		2.59	31.535°	
2.52	31.305°		3.00	31.58°	Foot out of calorimeter at 3.00.
2.53	31.34°				
2.54	31.39°		3.16	31.395°	Room temperature 26° C.
			Cooling of calorimeter 0.185° in 16 minutes.		

Volume of right foot in calorimeter (to upper level of external malleolus) 1,210 c.c.

Water equivalent of calorimeter and contents 4,807 c.c. Rectal temperature 37.5° C.

Here with the relatively high room temperature of 26.4° C. the flow per 100 cubic centimeters of foot per minute is 3.54 grams for the right foot, which alone was immersed.

On June 17, 1911, with a considerably lower room temperature (21.8° C.) and a somewhat lower calorimeter temperature, although a higher pulse rate, the flow was higher (4.93 grams for the right and 4.24 grams for the left foot).

June 17, 1911. Feet put into bath at 1.29 P.M. and into calorimeters at 1.40 P.M. 3,700 c.c. of water in each calorimeter. Room temperature 21.8° C. The examination was made in a very large room with the windows closed, so that the temperature was practically constant throughout, and was exactly 21.8° C. at the beginning and at the end of the experiment.

Time.	Temperature of calorimeters.		Time.	Temperature of calorimeters.		Notes.
	Right.	Left.		Right.	Left.	
1.39	30.20°	29.93°	1.49	30.58°	30.245°	
1.42	30.24°	29.98°	1.51	30.69°	30.37°	Better stirring.
1.43	30.28°	30.00°	1.52	30.73°	30.40°	
1.44	30.33°	30.04°	1.53	30.80°	30.43°	
1.45	30.37°	30.085°	1.54	30.84°	30.49°	
1.46	30.42°	30.10°	1.55	30.91°	30.52°	Feet out of calorimeters at 1.55.
1.47	30.46°	30.18°				
1.48	30.53°	30.225°	2.11	30.63°	30.27°	

Cooling of calorimeters in sixteen minutes, right 0.28° C., left 0.25°. Pulse (sitting) 84. Rectal temperature 37.6° C. Water equivalent of calorimeters with contents, right 4,777 c.c., left 4,714 c.c. Volume of right foot in calorimeter 1,170 c.c., of left 1,085 c.c. The subject was sitting, the feet hanging in the calorimeters.

On May 2, 1911, with an average room temperature of 25.2° C., the flow in the right foot was 3.57 grams, per 100 cubic centimeters per minute, which was only slightly increased (to 3.65 grams) on working the foot, in contrast to the marked increase obtained in the hand on rhythmically closing and unclosing the fist.

May 2, 1911. Right foot put into bath at 3.14 P.M., and into calorimeter at 3.26. The subject sat with his foot hanging in the calorimeter, which contained 5,000 c.c. of water. Left foot and leg not uncovered.

Time.	Temperature of right calorimeter.	Notes.	Time.	Temperature of right calorimeter.	Notes.
3.25½	30.26°	Room temperature 25.1°.	3.37	30.59°	
3.27	30.28°		3.38	30.625°	At 3.38 stopped working foot.
3.28	30.30°		3.39	30.66°	
3.29	30.32°	Room temperature 25.2°.	3.40	30.69°	
3.30	30.35°		3.41	30.71°	
3.31	30.39°		3.42	30.73°	
3.32	30.42°	Room temperature 25.3°.	3.43	30.765°	Room temperature 25.1°.
3.33	30.47°	At 3.33 began to work the foot.	3.44	30.79°	At 3.44 foot out of calorimeter.
3.34	30.49°		3.52	30.71°	
3.35	30.52°		3.53	30.70°	
3.36	30.56°	Room temperature 25.4°.	Volume of foot in calorimeter 1,220 c.c. Rectal temperature 37.4°. Water equivalent of calorimeter and contents, 6,115 c.c.		

Observations on emptying and filling the superficial veins in the hand and foot during such movements show that a greater influence may be expected to be exerted on the flow in the hand. This is confirmed by observations on the foot of C. B., in which the increase of flow during the movements (from 4.73 to 5.32 grams per 100 cubic centimeters of foot per minute), although greater than in the experiment on M. C., is still small in comparison with the increases formerly obtained in the hands.

C. B.—May 2, 1911. Right foot put into bath at 2.19 P.M., and into calorimeter at 2.30¾ P.M. 5,000 c.c. of water in the calorimeter. Pulse (sitting) 72. The subject sat during the blood flow measurements. The left foot and leg were not undressed. The right foot was hanging free in the calorimeter.

Time.	Temperature of right calorimeter.	Notes.	Time.	Temperature of right calorimeter.	Notes.
2.30	29.88°		2.41	30.24°	
2.32	29.91°		2.42	30.275°	At 2.42 stopped working foot.
2.33	29.94°				
2.34	30.00°		2.43	30.31°	Room temperature 26°.
2.35	30.03°	Room temperature 26.3°.	2.44	30.345°	
			2.45	30.39°	
2.36	30.06°		2.46	30.44°	At 2.46 foot out of calorimeter.
2.37	30.10°				
2.38	30.12°	Room temperature 26.3°.	2.56	30.34°	Room temperature 25.7°.
2.38⅛		Began to work the foot.	Volume of foot in calorimeter 980 c.c. Rectal temperature 36.9°. Mouth 36.45°.		
2.39	30.165°		Water equivalent of calorimeter and contents, 5,935 c.c.		
2.40	30.20°				

To compare the flow in the feet in the sitting and the supine position, which was necessary as a check on the observations in the fever patients, the experiments of March 26, 1913, on M. C., and of March 25, 1913, on J. R. were performed. In the first part of the experiment the subject reclined, with his feet in the calorimeters; then without altering the position of the feet in the least he sat up, the observations being continued without a break. In both experiments the soles of the feet rested throughout the observations on the footrest in the calorimeters. In the same experiments observations were made on the vasomotor reflexes elicited in one

foot by immersion of the other in warm or cold water, according to the routine already described for the hand.[6]

M. C.—March 26, 1913. Pulse (sitting) 98. Feet put into bath at 1.42 P.M. At 1.54 P.M. the subject lay down on his back. At 1.56 P.M. feet put into calorimeters. 2,775 c.c. of water in each calorimeter. The calorimeters as finally reduced in capacity were used.

Time.	Temperature of			Notes.
	Calorimeters.		Room.	
	Right.	Left.		
1.58	30.87°	30.88°	24.3°	
2.00	30.97°	30.93°	24.3°	
2.02	31.06°	31.01°		From 2.04 to 2.06 stirring of left calorimeter insufficient.
2.04	31.18°	31.14°	24.3°	
2.06	31.295°	31.20°		Pulse (lying down) 92.
2.08	31.43°	31.39°	24.3°	
2.10	31.57°	31.51°		
2.12	31.70°	31.65°	24.1°	
2.14	31.795°	31.75°		At 2.14 subject sat up and continued sitting for rest of experiment.
2.16	31.87°	31.82°	24.2°	
2.34		32.38°	24.6°	Subject says the left foot feels colder than before.
2.36		32.42°	24.6°	
2.38		32.465°	24.6°	
2.40		32.495°		
2.42		32.54°	24.6°	
2.44		32.60°		
2.46		32.68°	24.6°	
2.48		32.73°		
2.50		32.78°		At 2.50 right foot put in water at 43°.
2.52		32.80°	24.6°	
2.54		32.86°		

Time.	Temperature of			Notes.
	Calorimeters.		Room.	
	Right.	Left.		
2.18	31.92°	31.91°	24.3°	
2.20	31.99°	31.96°		
2.22	32.08°	32.04°	24.6°	
2.24	32.10°	32.10°		Stirring of right calorimeter insufficient for the last minute.
2.26	32.18°	32.18°	24.6°	
2.28	32.23°	32.25°		
2.30	32.295°	32.31°	24.4°	At 2.30 right foot put in water at 8° C.
2.32		32.37°		
2.32½	32.32°			
2.56		32.905°	24.6°	
2.58		32.99°		
3.00		33.095°	24.7°	
3.02		33.22°		
3.03				Temperature of right calorimeter 31.80°.
3.04		33.31°	24.6°	
3.06		33.405°		At 3.06 foot removed from calorimeter.
3.07		33.40°		
3.18		33.17°		Pulse (sitting) 97°.

Cooling of right calorimeter 0.52° in 31½ minutes.
Cooling of left 0.23° in 11 minutes.
Volume of right foot 1,232 c.c., of left 1,195 c.c.

[6] Stewart, G. N., *Heart, loc cit.*, p. 76.

Hands put into bath at 3.40 P.M. Hands put into calorimeters at 3.52. 3,015 c.c. of water in each calorimeter.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Notes.
3.51½	31.10°	31.22°		
3.53	31.10°	31.26°		
3.54	31.18°	31.32°	24.3°	
3.55	31.26°	31.41°		
3.56	31.35°	31.50°		
3.57	31.44°	31.59°		
3.58	31.53°	31.67°	24.3°	
3.59	31.63°	31.76°		
4.00	31.72°	31.81°	24.3°	
4.01	31.795°	31.895°		
4.02	31.87°	31.98°		At 4.02 left hand put in water at 8° C.
4.03	31.91°		24.3°	
4.04	31.98°			
4.05	32.02°		24.4°	
4.06	32.08°			
4.07	32.14°		24.5°	
4.08	32.21°			
4.09	32.27°		24.3°	
4.10	32.31°			

Time.	Temperature of Right calorimeter.	Room.	Notes.
4.11	32.37°		
4.12	32.43°	24.4°	
4.13	32.49°		
4.14	32.53°	24.3°	
4.15	32.595°		
4.16	32.67°		At 4.16 left hand
4.17	32.71°	24.4°	put in water at
4.18	32.74°		43° C.
4.19	32.78°		
4.20	32.83°	24.25°	
4.21	32.88°		
4.22	32.93°	24.35°	
4.23	32.98°		
4.24	33.03°	24.3°	
4.25	33.08°		
4.26	33.125°	24.3°	
4.27	33.22°		At 4.27 hand out of calorimeter.
4.53	32.90°		Temperature of left calorimeter 31.40°.

Cooling of calorimeters, right 0.32° C. in 26 minutes; left 0.58° C. in 51 minutes. Volume of right hand 505 c.c., of left 485 c.c. Rectal temperature 37.1° C. Water equivalent of calorimeters with contents in feet observations, right 3,899, left 3,871. The corresponding numbers for the hand observations are, right 3,499, and left 3,483.

J. R.—March 25, 1913. Feet in bath at 1.44 P.M., and in calorimeters at 1.58 P.M. Pulse (sitting) 85. At 2.01½ P.M. subject lay on his back. 2,775 c.c. of water in each calorimeter, as finally reduced in capacity.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Notes.
1.57	30.86°	30.77°		
2.00	30.87°	30.81°		
2.02	30.98°	30.88°		
2.04	31.15°	31.06°	24.8°	
2.06	31.30°	31.19°	25.0°	
2.08	31.48°	31.365°		
2.10	31.66°	31.495°	25.3°	
2.12	31.82°	31.68°		Pulse (lying) 76
2.14	31.95°	31.79°	24.9°	
2.16	32.095°	31.91°	24.7°	Sat up at 2.16
2.18	32.23°	32.03°	24.6°	and continued sitting

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Notes.
2.20	32.33°	32.12°	24.6°	
2.22	32.44°	32.20°	24.7°	
2.24	32.58°	32.33°		
2.26	32.72°	32.44°	24.6°	
2.28	32.85°	32.54°	24.6°	
2.30	32.97°	32.65°	24.4°	
2.32	33.02°	32.71°	24.5°	
2.34	33.095°	32.77°		At 2.34½
2.36	33.10°	32.81°		right foot
2.38		32.85°		put in water at 10° C.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Notes.
2.40		32.885°	24.5°	
2.42		32.92°		
2.44		32.97°	24.5°	At 2.44 right foot put in water at 43° C.
2.46		33.025°	24.5°	
2.48		33.095°	24.4°	
2.50		33.13°	24.4°	
2.52		33.195°	24.4°	
2.54		33.28°	24.5°	
2.56		33.37°		
2.58		33.445°	24.4°	
3.00		33.505°	24.4°	Foot removed from calorimeter at 3.00.
3.03		33.50°		Temperature of right 32.61° C.
3.26		33.04°		Temperature of right 32.23° C.

Cooling of calorimeters, right 0.87° C. in 50 minutes (0.38° in the last 23 minutes); left 0.46° in 23 minutes. Volume of right foot 1,008 c.c., of left foot 959 c.c.

Hands in bath at 3.30 P.M. At 3.42½ hands in calorimeters. 3,015 c.c. of water in each calorimeter.

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Notes.
3.41	31.22°	31.24°		
3.43	31.19°	31.26°		
3.44	31.22°	31.30°	24.7°	
3.45	31.295°	31.36°	24.9°	
3.46	31.35°	31.40°		
3.47	31.40°	31.43°	24.95°	
3.48	31.44°	31.465°		
3.49	31.51°	31.495°		
3.50	31.57°	31.53°		
3.51	31.65°	31.58°	24.9°	
3.52	31.71°	31.62°		
3.53	31.76°	31.65°	24.8°	Here the cold water was mentioned.
3.54	31.795°	31.68°		
3.55	31.84°	31.695°	24.6°	
3.56	31.89°	31.73°		
3.57	31.95°	31.76°	24.3°	Here the cold water was brought.
3.58	31.98°	31.78°		
3.59	32.02°	31.79°		At 3.59 left hand put in water at 7.8° C.
4.00	32.07°		24.4°	
4.01	32.08°			

Time.	Temperature of Right calorimeter.	Room.	Time.	Temperature of Right calorimeter.	Room.	Time.	Temperature of right calorimeter.	Notes.
4.02	32.095°	24.4°	4.11	32.36°		4.20	32.82°	Hand removed from calorimeter at 4.20.
4.03	32.11°		4.12	32.38°	24.4°			
4.04	32.13°	24.4°	4.13	32.40°		4.34	32.67°	Temperature of left calorimeter at 4.34 is 31.41°.
4.05	32.155°		4.14	32.44°	24.6°			
4.06	32.195°	24.4°	4.15	32.49°				
4.07	32.225°		4.16	32.56°				
4.08	32.265°		4.17	32.61°	24.4°			Cooling of right calorimeter 0.15° in 14 minutes.
4.09	32.295°	24.3°	4.18	32.66°				Cooling of left calorimeter 0.38° in 35 minutes.
4.10	32.33°		4.19	32.72°	24.5°			Volume of right hand 393 c.c.
								Volume of left hand 376 c.c.
At 4.10 left hand put in water at 43.1°								Rectal temperature 37.0° C.

Water equivalent of calorimeters with contents in the feet observations, right 3,731, left 3,694; in the hand observations, right 3,411, left 3,396.

In M. C. the flow in the right foot was 5.11 grams, and in the left 5.23 grams per 100 cubic centimeters of foot per minute lying down, and only 3.96 grams for the right, and 4.17 grams for the left sitting up. In J. R. there was also a reduction in the flow in the sitting position, although less marked than in M. C. (from 7.98 and 7.58 grams for the right and left foot, respectively, to 7.84 and 6.90 grams per 100 cubic centimeters of foot per minute). This is of importance in connection with the fever observations for it permits the assumption that the observed flows were at any rate as large as would have been obtained had the patients been sitting. These observations can therefore be safely compared with all the data obtained on normal persons. It will be noticed that the flows in M. C. in the experiment of March 26, 1913, agree with the results obtained nearly two years previously. This shows that when proper precautions are taken, the range of variation in the observed foot flow in one and the same healthy person is moderate, just as is the case for the hands. Therefore, when great differences, usually in the direction of deficiency, are found in pathological cases it can be assumed with some confidence that they are not due to accidental differences in the external conditions.

The reflex vasomotor effects from one foot to the other are similar to those obtained in the hands, only the constriction elicited by cold seems to be more durable. Thus in M. C. immersion of the right foot in water at 8° C. caused a diminution in the flow in the left foot from 4.17 to 3.12 grams per 100 cubic centimeters per minute for the first ten minutes of the immersion. This gave place rather suddenly, as in the hand, to an increase in the flow (to 5.17 grams per 100 cubic centimeters per minute) for the remaining eight minutes of the immersion. The greater susceptibility to vasoconstriction of the feet than of the hands has already been mentioned in connection with the technique of the measurement since it introduces a possibility of error which must be carefully minimized or excluded, especially in pathological cases, for in not a few clinical conditions this susceptibility seems to be augmented. Where organic local changes are excluded a marked diminution in the foot flow due to vasoconstriction can usually be discriminated from

a feeble flow in the feet due to deficient driving power in the circulation, by comparing the flow in the hands under the same conditions. For M. C. this was done in the experiment under discussion. The flow in the hands in the sitting posture came out 14.71 grams per 100 cubic centimeters per minute for the right, and 15.10 grams for the left. Even the greatest foot flow seen in this individual does not approach one half of the hand flow per 100 cubic centimeters of tissue per minute. The average of four experiments on different dates for the right foot in the sitting position is 4.28 grams. The average of nine already published[7] experiments on the hands of M. C. in the sitting position, suitable as regards the range of room temperature and comparable in other respects, is 12.77 grams for the right hand, and 12.29 grams for the left.

In table I, at the end of the following paper,[8] are given the condensed results of nine additional experiments on M. C.'s hands, the average flow in which is 12.95 grams for the right hand, and 12.41 grams for the left. If the observations of May 24, 1911, and November 27, 1911, be excluded, as obviously extreme results due to external temperatures which for the conditions of our experiments may be considered extreme, we get an average of 12.91 grams for the right hand, and 12.61 grams for the left.

In this man, then, the ratio of 1 to 3 holds approximately for the hand and foot flow as determined under our conditions. It is not pretended that the deficiency in the foot flow as compared with the hand flow is in reality exactly as great as this. As already pointed out, the difference between the observed and the actual flow in the foot may be expected to be greater than the corresponding difference for the hand on account of the difference in the dimensions of the two parts. The smaller the foot the closer must the observed come to the actual flow. Yet the error due to this is apparently not great, as is indicated by the comparison of the hand and foot flows in the young boy, N. R. The observations on him were made for the purpose of testing this point. The volume of

[7] Stewart, G. N., *Heart, loc. cit.*, p. 33.

[8] Stewart, G. N., *Jour. Exper. Med., loc. cit.*, table I, p. 385.

his feet is no greater than that of M. C.'s hands, in fact somewhat less, and the average thickness of the skin even including that of the soles is probably less. Although the greatest thickness of the boy's foot is probably somewhat greater than the maximum thickness of M. C.'s hand, the dimensions of the two objects do not differ sufficiently to forbid the assumption that the equalization of temperature of the boy's foot and the man's hand with that of the calorimeter would be about equally complete. If then the flow in the adult foot as estimated in this method were much below the actual flow, the measured flow in the boy's foot should approach much nearer to that in his hand than is the case in the adult. That this is not so is shown in the experiment of April 8, 1913.

N. R., a healthy boy aged 8 years and 9 months. Hebrew. Pulse (sitting) 84. Blood flow measured in feet and hands. Feet in bath at 2.03 P.M., in calorimeters at 2.17½ P.M., and out of calorimeters at 2.55. He sat throughout the examination. 3,015 c.c. of water in each foot calorimeter, as finally reduced in capacity. An additional footrest was used to bring the flange of the calorimeter to the same level on the ankle as in the observations with adults. His legs were loosely wrapped up with towels down to the calorimeters.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.13	30.87°	30.89°	23.5°	2.39	30.92°	30.85°	23.95°
2.19	30.80°	30.80°	24.2°	2.41	30.93°	30.86°	23.95
2.21	30.80°	30.80°	24.3°	2.43	30.96°	30.87°	24.0°
2.23	30.795°	30.795°	24.3°	2.45	30.965°	30.875°	24.0°
2.25	30.805°	30.80°	24.3°	2.47	30.99°	30.88°	24.0°
2.27	30.82°	30.805°		2.49	31.02°	30.91°	24.0°
2.29	30.825°	30.81°	24.1°	2.51	31.04°	30.915°	24.0°
2.31	30.85°	30.81°	24.0°	2.53	31.05°	30.92°	24.1°
2.33	30.87°	30.82°	24.0°	2.55	31.07°	30.925°	
2.35	30.89°	30.82°	24.0°	2.57	31.08°	30.89°	
2.37	30.90°	30.825°	23.95°	3.34	30.58°	30.39°	

Cooling of calorimeters in 37 minutes, right 0.50°, left 0.50°. Volume of right foot 491 c.c., of left foot 480 c.c. Water equivalent of calorimeters with contents, right 3,593, left 3,585.

Hands in bath at 3.26 P.M., in calorimeters at 3.37½ P.M., and out of calorimeters at 3.58. 3,115 c.c. of water in each hand calorimeter.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.37	30.82°	30.98°		3.49	30.94°	31.105°	
3.39	30.82°	30.96°	23.0°	3.50	30.96°	31.11°	24.0°
3.40	30.83°	30.98°	23.9°	3.51	30.965°	31.11°	
3.41	30.835°	30.99°		3.52	30.975°	31.125°	24.0°
3.42	30.84°	30.995°	23.85°	3.53	30.99°	31.135°	
3.43	30.845°	31.00°		3.54	31.00°	31.145°	24.1°
3.44	30.85°	31.015°	23.9°	3.55	31.00°	31.15°	
3.45	30.88°	31.04°		3.56	31.02°	31.175°	
3.46	30.90°	31.06°	23.95°	3.57	31.04°	31.195°	24.1°
3.47	30.915°	31.095°		3.58	31.095°[9]	31.19°[9]	
3.48	30.935°	31.10°	24.0°	4.14	30.95°[9]	31.00°[9]	
				Pulse (sitting) 80. Rectal temperature 37.2°.			

Cooling of calorimeters in 16 minutes, right 0.145°, left 0.19°. Volume of right hand 206 c.c., of left hand 180 c.c. Second observation on hand volumes, right hand 200 c.c., left 182 c.c. Average for right 203 c.c., for left 181 c.c. Water equivalent of hand calorimeters with contents, right 3.357, left 3.340.

In this experiment the blood flow in the feet for the last eighteen minutes of the immersion in calorimeters was 3.37 grams per 100 cubic centimeters of foot per minute for the right foot, and 2.86 grams for the left. For the hands for the last 15 minutes in the calorimeters the flow was 8.22 grams per 100 cubic centimeters per minute for the right hand, and 8.95 grams for the left. Considering only the last ten minutes in the calorimeters, the flow comes out 8.08 grams per 100 cubic centimeters per minute for the right hand, and 7.75 grams for the left. In round numbers the hand flow (average of the two hands) is 8 grams per 100 cubic centimeters of tissue, and the foot flow (average of the two feet) 3 grams per 100 cubic centimeters of tissue, practically the same ratio as in M. C. The smaller calculated flow in the foot than in the hand of the adult is therefore not due to more complete equalization of the temperature of the hand with that of the water than is the case for the foot.

In C. B. in the experiment of May 2, 1911, the flow in the right foot was 4.73 grams per 100 cubic centimeters per minute, with room temperature a little over 26° C., whereas the highest flow ob-

[9] These readings were all verified.

served in his hands on an extremely hot day, with room temperature 28.9°, was 12.54 grams for the right and 13.34 grams for the left (May 22, 1911), and the average of all hand observations on this man works out at 8.4 grams per 100 cubic centimeters per minute. The ratio of the volume of C. B.'s foot to M. C.'s is about 1 to 1.25.

In the experiment of March 25, 1913, on J. R., comparison is complicated by the fact that the left hand has a much smaller flow than the right (12.38 grams per 100 cubic centimeters per minute for the right, 8.82 grams for the left). A difference as great as this is rare in normal persons. In fact, in those I have examined there is no other instance, although in clinical cases with unilateral anatomical differences and even without such in certain neurological conditions, still greater differences may be found. Where a difference of any magnitude is temporary, that is to say, where it is obtained one day and not the next, or the next week, it cannot be far wrong to interpret it as due to such an accidental vasomotor difference as is familiar in other parts; for example, when one ear is hot and red and the other cold and pale. In J. R., however, the same difference between the hands was found on March 22, 1913, 10.08 grams per 100 cubic centimeters per minute for the right hand, and 7.25 grams for the left, with room temperature 24.0° C. The ratio between the flows in the left and right hands is the same (1 to 1.39) as on March 25, when it was 1 to 1.40.

In the absence of further examination, which will be made later, all that can be said is that this indicates some permanent condition on which the inequality of the flow in the two hands depends. The young man states that he feels well, but he bleeds easily. A week before the examination of March 22 he lost half a glass of blood by bleeding from the nose. A small cut on the finger bled for fifteen minutes a few days after. He says that cuts always bleed a long time. He states that a brother and a sister bleed easily and the bleeding is difficult to stop. Some of his cousins also have this peculiarity. The slow pulse rate, not responding to change of posture, on March 22, and the much more rapid rate on March 25 are also peculiar features, and it is possible that this case should not

be considered entirely a normal one. At any rate, for our purpose it will be safest to neglect the left hand. The ratio between the flows in the right foot and right hand in the sitting posture then comes out approximately 1 to 1.6. The ratio of the combined volume of his feet to the combined volume of the feet of M. C. is 1 to 1.28.

J. R.—March 22, 1913. Hands in bath at 11.55 A.M., in calorimeters at 12.06½ P.M. 3,015 c.c. of water in each calorimeter. Pulse (sitting) 52, counted for a minute at the end of the examination. Just before the examination of the hands the rate was 52 and 51 for two successive minutes in the supine position.

Time.	Temperature of			Notes.	Time.	Temperature of			Notes.
	Calorimeters.		Room.			Calorimeters.		Room.	
	Right.	Left.				Right.	Left.		
12.06	30.69°	30.75°			12.16	30.91°	30.89°	24.0°	
12.08	30.59°	30.71°			12.17	30.94°	30.91°		
12.09	30.60°	30.715°	22.7°	Radiator turned on.	12.18	30.99°	30.93°	24.1°	
					12.19	31.04°	30.97°	24.2°	
12.10	30.605°	30.72°	22.9°		12.20	31.10°	30.995°		
12.11	30.65°	30.745°	22.9°		12.21	31.125°	31.00°	24.3°	
12.12	30.695°	30.77°	22.95°		12.22	31.18°	31.03°		At 12.22 he saw the cold water.
12.13	30.73°	30.78°							
12.14	30.79°	30.80°	23.8°						
12.15	30.83°	30.83°	23.7°		12.23	31.20°	31.04°		At 12.23 left hand in water at 8° C.
					12.24	31.235°			
12.25	31.25°		24.3°		12.38	31.425°		23.95°	
12.26	31.26°		24.3°		12.39	31.46°			
12.27	31.27°				12.40	31.49°			
12.28	31.28°		24.2°		12.41	31.52°		23.9°	
12.29	31.295°				12.42	31.58°			
12.30	31.31°				12.43	31.62°		23.85°	
12.31	31.325°		24.0°		12.44	31.68°			
12.32	31.345°				12.45	31.75°		24.0°	
12.33	31.38°		24.0°		12.46	31.795°			
12.34	31.39°				12.47	31.84°		24.1°	
12.35	31.40°				12.48	31.92°			Hand out of calorimeter at 12.48.
12.36	31.41°		23.95°	At 12.36 left hand in water at 43.2°.					
12.37	31.42°								
					1.01	31.80°	30.64°		

Cooling of calorimeters, right 0.12° C. in 13 minutes, left 0.40° C. in 38 minutes. Volume of right hand 397 c.c., of left hand 354 c.c. Water equivalent of calorimeters with contents, right 3,412, left 3,378. Rectal temperature 36.5° C.

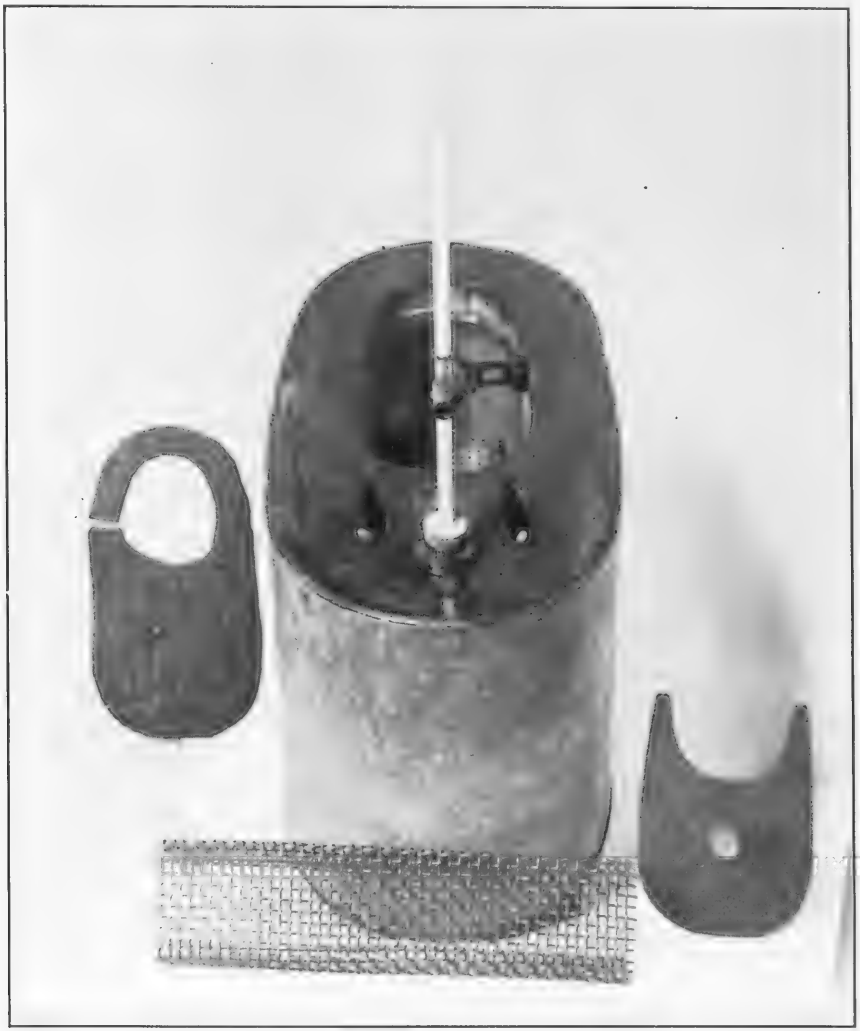

FIG. 1.

(Stewart: Studies on the Circulation in Man.

FIG. 2.

(Stewart: Studies on the Circulation in Man.)

FIG. 3.

(Stewart: Studies on the Circulation in Man.

It will, therefore, probably not be far wrong to assume that in normal adults the ratio between the foot flow and the hand flow as estimated in this way will not pass much in either direction beyond the limits 1 to 3 and 1 to 2. Where we encounter foot flows which, in the absence of known anatomical causes, obliterative arteritis for example, are much below one third of the hand-flow in the same person, we shall usually not be wrong in concluding that the peripheral resistance in the path through the feet is increased in proportion to that of the path through the hand, and that in general the increase is due to vasomotor influence.

CONCLUSIONS.

1. The blood flow in the feet is smaller per unit of volume of the part than in the hand, the ratio of foot flow to hand flow per 100 cubic centimeters of the part usually lying in normal persons between 1 to 3 and 1 to 2.

2. In the supine position, with the legs hanging down, the flow in the feet seems to be somewhat greater than in the sitting position.

EXPLANATION OF PLATES.

PLATE 22.

FIG. 1. Foot calorimeter. For description see text (p. 354).

PLATE 23.

FIG. 2. Arrangement for the measurement of foot flow with the patient in sitting position. At the left is shown the arrangement for measuring the volume of the feet. The legs should be more completely covered.

PLATE 24.

FIG. 3. Arrangement for measuring the foot flow in a patient not well enough to sit up. The legs should be more completely covered than is indicated in the figure.

STUDIES ON THE CIRCULATION IN MAN.

VIII. The Blood Flow in the Feet with Special Reference to Fever.*

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In spite of much discussion we possess but little exact knowledge of the changes in the cutaneous circulation in fever, which constitute an essential, probably the chief, factor in the derangement of the temperature-regulating mechanism. I have endeavored to make a small contribution to our data by measuring approximately the blood flow in the feet in a number of cases of typhoid fever and in one case of pneumonia. Of one of the cases of typhoid four examinations were made in the course of a month. In a second case a similar program was planned but had to be modified on account of hemorrhage, and only two examinations were carried out. These, however, happened to be at the most interesting stages in the course of the fever. For comparison three cases at different stages in convalescence were investigated, each on one occasion. In the pneumonia case two examinations were secured, the last one four days before the death of the patient. The first of the typhoid patients was far enough advanced in convalescence to permit him to sit up in a chair for the examination of the hands.

F. D.—A sailor, aged 26 years, height 5 feet 9½ inches, was admitted to the City Hospital on September 5, 1912, suffering from typhoid fever. About a month before he fell into the Cuyahoga River and swallowed water. The drinking water on the lake boats on which he works is, according to him, far from being beyond suspicion, as it is taken from the lake. His maximum temperatures were as follows:

September 5,	104.0° F.	September 10,	104.0° F.
September 11,	103.4° F.	September 12,	102.4° F.
September 15,	100.0° F.	September 17,	98.6° F.

* Received for publication, April 30, 1913.

Only one examination of the blood flow was obtained, and that was when he was so far advanced in convalescence that he walked into the room. The foot flow was measured, as in the other cases, while he lay on his back on the movable bed.

October 1, 1912. Feet put into bath at 1.21 P.M., and into calorimeters at 1.38½ P.M. 3,740 c.c. of water in each calorimeter. Pulse (lying on back) 81.

Time.	Temperature of Calorimeters. Right.	Left.	Room.
1.37	31.19°	31.16°	
1.42	31.02°	30.95°	22.1°
1.44	30.99°	30.94°	
1.46	30.97°	30.92°	21.8°
1.48	30.945°	30.89°	22.1°
1.50	30.935°	30.87°	
1.52	30.92°	30.86°	22.3°
1.54	30.91°	30.845°	
1.56	30.90°	30.835°	22.5°
1.58	30.895°	30.815°	22.45°
2.00	30.89°	30.80°	
2.02	30.88°	30.79°	22.45°
2.04	30.875°	30.78°	22.45°
2.06	30.87°	30.77°	
2.08	30.82°	30.73°	
2.18	30.66°	30.565°	22.45°

Cooling in 10 minutes of right calorimeter 0.16° C., of left 0.165°. Volume of right foot 1,135 c.c., of left 1,117 c.c. Feet out of calorimeters at 2.06½.

Immediately afterward the flow in the hands was measured. Hands put into bath at 2.32 P.M., and into calorimeters at 2.42¼ P.M. 3,015 c.c. of water in each calorimeter. Pulse (sitting) 90. Blood pressure, left arm, systolic 106 (palpation), 107 (stethoscope); 82 (sound gone). Another observation, systolic 109, 85 (sound gone).

Time.	Temperature of Calorimeters. Right.	Left.	Room.	Time.	Temperature of calorimeters. Right.	Left.	Notes.
2.41	31.71°	31.69°	22.9°	2.50	31.97°	31.94°	Room temperature 22.8°.
2.43	31.70°	31.70°		2.51	31.99°	31.96°	
2.44	31.76°	31.74°	23.2°	2.52	32.02°	31.985°	Room temperature 22.8°.
2.45	31.80°	31.77°		2.53	32.06°	32.02°	Hands out of calorimeters at 2.53.
2.46	31.84°	31.81°	23.1°	3.07	31.84°	31.81°	
2.47	31.88°	31.85°					
2.48	31.91°	31.88°					
2.49	31.935°	31.905°					

Cooling in 14 minutes of right calorimeter 0.22°, of left 0.21°. Volume of right hand 425 c.c,. of left 423 c.c. Patient is ambidextrous. Rectal temperature, 37.2°. Water equivalent of right calorimeter and contents 3.435; of left and contents 3.434.

Here the hand flow lies within the normal range, being 9.16 grams per 100 cubic centimeters per minute for the right hand, and 8.63 grams for the left, with room temperature 23° C. The foot flow is much below the normal (0.98 grams per 100 cubic centimeters per minute for the right, and 0.85 grams for the left). The ratio of the combined foot flows to the combined hand flows (1 to 9.7)

is much too small and indicates clearly an abnormal sensitiveness of the feet to vasoconstriction, the exciting cause being probably the necessary exposure of the feet to the air and water. We shall see that there is evidence in the foot flows of abnormally great cutaneous vasoconstriction in all the typhoid cases examined, and at all stages of the fever which happened to coincide with an examination. This tendency to vasoconstriction seems to be carried into convalescence.

In the case of J. McD., who was still further advanced in convalesence than F. D., the prolonged reflex vasoconstriction, not followed by any vasodilatation in the left hand when the right was immersed in warm water, might be interpreted in the same way, as also the marked inferiority of the flow in the right hand as compared with the left. Such differences between the two hands in the absence of any known anatomical cause are apt to be observed in persons who exhibit other evidences of nervous, including vasomotor, instability.

J. McD.—A laborer, aged 26 years, was admitted to the City Hospital on April 11, 1912, suffering from typhoid fever. The maximum temperature reached was 105.6° F. on April 12 and April 13. The opportunity to examine his blood flow was not offered until he was convalescent. Temperature at 4 A.M. on May 16, 98.5° F. He is emaciated and his appetite is ravenous.

The blood flow in the hands was examined on May 16, 1912. Hands in bath at 1.47 P.M., and in calorimeters at 1.56½ P.M. 3,015 c.c. of water in each calorimeter. Mouth temperature 36.7° C. Pulse (sitting) 80.

Time.	Temperature of calorimeters.		Notes.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
1.56	30.92°	31.11°		2.07	31.28°	31.52°	Room temperature 23.6°.
1.58	30.94°	31.12°	Room temperature 22.6°	2.08	31.315°	31.555°	
1.59	30.97°	31.15°		2.09	31.37°	31.625°	Ears red.
2.00	31.00°	31.20°		2.10	31.405°	31.66°	Room temperature 23.2°.
2.01	31.04°	31.23°	Room temperature 23.1°	2.11	31.44°	31.72°	
2.02	31.08°	31.27°		2.12	31.48°	31.75°	
2.03	31.10°	31.31°		2.13	31.52°	31.81°	
2.04	31.14°	31.35°		2.14	31.56°	31.84°	Room temperature 23.1°.
2.05	31.19°	31.41°	Room temperature 23.2°	2.15	31.59°	31.90°	
2.06	31.22°	31.455°		2.16	31.62°	31.93°	At 2.16 right hand put in water at 8°. Room temperature 23.0°.

Time.	Temperature of calorimeters.		Notes.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
2.17		31.945°		2.28		32.26°	
2.18		31.96°	Room temperature 23.2°	2.29		32.305°	Room temperature 23.0°.
2.19		31.99°		2.30		32.33°	
2.20		32.03°		2.31		32.35°	Room temperature 23.1°.
2.21		32.055°					
2.22		32.08°		2.32		32.38°	
2.23		32.12°	Room temperature 23.2°	2.33		32.41°	
				2.34		32.425°	Room temperature 23.6°.
2.24		32.155°	Right hand in the cold water is redder than is usually the case	2.35		32.435°	
				2.36		32.455°	Hand out of calorimeter at 2.36. Room temperature 23.7°.
2.25		32.20°					
2.26		32.23°	Right hand put in water at 42.9°	2.44		32.35°	Temperature of right calorimeter 31.29°.
2.27		32.24°	Room temperature 23.0°	Cooling of right calorimeter in 28 minutes, 0.33°; of left in 8 minutes, 0.105°.			

Volume of right hand 511 c.c., of left hand 505 c.c. His hands are large although they have lost a considerable proportion of the soft tissues.

In this experiment the flow for a period of eighteen minutes before the vasomotor reaction was tested was 6.95 grams per 100 cubic centimeters per minute for the right hand and 8.42 grams for the left. During the first five minutes of immersion of the right hand in cold water the flow in the left was reduced to 6.10 grams per 100 cubic centimeters per minute and rose again to 8.04 grams for the next five minutes of immersion. When the right hand was put into warm water the flow in the right fell to 6.25 grams per 100 cubic centimeters per minute for the whole period of immersion (ten minutes).

Hyperexcitability of the vasoconstrictor mechanisms of the periphery is most naturally interpreted as a defensive reaction of the organism by which an increased supply of blood is afforded for those internal organs which bear the brunt of the infection. The fact that the blood pressure is low and the pulse dicrotic in spite of the constriction of the vessels of the periphery, indicates that other vascular paths, especially the splanchnic area, are more widely

opened than usual. The fever, the rise of temperature of the internal parts and of the blood, may be considered as, in the main, a secondary and inevitable, and perhaps in itself an unimportant consequence of a sustained cutaneous vasoconstriction. When the temperature has risen to a certain height a balance between heat production and heat loss is necessarily again reached in spite of the vasoconstriction. Indeed, were the circulation in the skin entirely stopped it is clear that such a balance must also be eventually reached by augmentation of the flow of heat by conduction from the deeper parts.

It must be remembered that with a normal flow through the skin in fever the amount of heat given off would be greater than normal on account of the higher temperature of the blood. Table I illustrates this point. Thus while the amount of heat given off per 100 cubic centimeters of foot per minute in all the fever cases does not rise beyond the range for the normal persons, the amount of heat given off per gram of blood passing through the foot is much greater in fever than with normal body temperature. And it is precisely when the flow of blood is small and the rectal temperature high that the heat loss per gram of blood is greatest. Thus in J. B. on October 16, 1912, with rectal temperature 40.2° C., and in M. V. on October 8, 1912, with rectal temperature 40.5° C., over eight small (gram) calories per gram of blood are given off to the calorimeters, whereas the corresponding number for the normal persons is always below six for the average of the two feet. A glance at table II will show that the difference does not depend upon variations in the calorimeter temperatures in the different experiments. It is essential to point this out as it is obvious that it is inherent in the method that the lower the calorimeter temperature the greater must be the heat loss per gram of blood passing through the part. The slight differences in the heat given off by the two hands or by the two feet in one and the same experiment depend upon differences in the calorimeter temperatures.

If the cutaneous vasoconstriction is in the interests of an increase in the blood flow to the organs on which the stress of the infection falls, it is plain that if the increased temperature is to be combatted by direct measures the most rational procedure is to hasten the loss

of heat without dilatation of the skin vessels, or still better while their constriction is even increased. This indication is admirably met by the cold bath treatment. On the other hand, the use of antipyretics which act by causing cutaneous vasodilatation would seem to be unphysiological since in diminishing the temperature they cause the withdrawal of blood from the seats of infection.

F. S.—A laborer, aged 28 years, was admitted to the City Hospital on September 16, 1912, suffering from typhoid fever. He became ill a week before admission, had two chills, and vomited twice. Pulse dicrotic, low tension. The maximum temperatures were as follows:

September 17,	104.4° F.	September 19,	104.0° F.
September 20,	104.0° F.	September 21,	104.0° F.
September 24,	103.0° F.	September 26,	102.0° F.
October 1,	100.0° F.		

It was only possible to examine the blood flow once and that was during convalescence.

The blood flow in the feet was examined on October 2, 1912. Pulse (reclining on back) 72. Feet in bath at 2.43 P.M., in calorimeters at 2.56½ P.M. 3,740 c.c. of water in each calorimeter. Blood pressure, right arm, systolic 106 (palpation), 104 (stethoscope); 77 (sudden diminution of sound), 58½ (disappearance of sound). Rectal temperature 37.9° C.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.55	31.77°	31.69°		3.16	31.795°	31.59°	24.15°
2.59	31.71°	31.55°	23.9°	3.18	31.80°	31.60°	24.2°
3.01	31.71°	31.55°	23.9°	3.20	31.805°	31.61°	24.15°
3.03	31.725°	31.565°	23.95°	3.22	31.81°	31.625°	24.1°
3.05	31.73°	31.57°	24.1°	3.24	31.825°	31.63°	
3.07	31.75°	31.575°		3.26	31.795°	31.595°	
3.09	31.77°	31.585°	24.15°	3.44	31.52°	31.325°	
3.11	31.78°	31.59°	24.2°				
3.13	31.78°	31.585°	24.2°				

Cooling of right calorimeter 0.275° in 18 minutes, of left 0.27°. Volume of right foot 1,295 c.c., of left foot 1,258 c.c. Feet out of calorimeters at 3.24½.

The patient was examined about a fortnight after admission and ten days after the fastigium. The flow was distinctly below normal, in the right foot 1.47 grams per 100 cubic centimeters per minute; in the left 1.39 grams, the room temperature being 24.1° C.

M. V.—A laborer, aged 29 years, height about 6 feet, was admitted to the City Hospital on October 7, 1912, eight days after he became ill with typhoid. He was quite ill on admission. Rose spots. Maximum temperature October 7 at 10 P.M. 105.6° F. Temperature October 8 at 5 A.M. 103.4° F. He had a full bath on October 7 and another on October 8 about 1 P.M.

First Examination of Blood Flow.—October 8, 1912. Feet in bath at 3.01 P.M., and in calorimeters at 3.18 P.M. 3,740 c.c. of water in each calorimeter. Pulse (lying on back) 108. Blood pressure, right arm, systolic 118, 89 (sound entirely gone). Rectal temperature 40.5° C.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
3.13	31.06°	30.93°	22.3°	3.34	30.95°	30.88°	22.1°
3.20	30.89°	30.74°	22.4°	3.36	31.01°	30.91°	22.15°
3.22	30.86°	30.72°	21.8°	3.38	31.03°	30.945°	22.2°
3.24	30.84°	30.71°	21.9°	3.40	31.095°	30.98°	
3.26	30.835°	30.74°	22.1°	3.42	31.04°	30.95°	
3.28	30.86°	30.755°	22.2°	3.59	30.79°	30.695°	
3.30	30.88°	30.79°	22.15°	Cooling of right calorimeter 0.25° in 17 minutes, of left 0.255°. Volume of right foot 1,322 c.c., of left, 1,294 c.c. Feet out of calorimeters at 3.40.			
3.32	30.92°	30.85°	22.15°				

Second Examination.—November 5, 1912. The patient says that he is now feeling well. The maximum and minimum temperatures were as follows:

October 9, 105.0° and 102.6° F. October 10, 104.8° and 103.0° F.
October 11, 104.0° and 102.0° F. October 14, 105.0° and 101.0° F.
October 17, 104.6° and 102.0° F.

On October 17 he had a hemorrhage from the bowels, and on October 18 a hemorrhage from one ear. On October 30 the maximum temperature was still 104.0° F., the minimum 100.0°. From that date the temperature steadily diminished to 101.4° (maximum) and 98.4° (minimum) on November 4.

Feet in bath at 2.43 P.M. (lying on back on the ambulance). Pulse 80. Feet in calorimeters at 2.54 P.M. 2,775 c.c. of water in each calorimeter. The capacity of the calorimeters had been reduced since the last examination. The volumes of the feet are therefore not strictly comparable in the two experiments. Rectal temperature 37.95° C. Blood pressure, right arm, systolic 107, 62 (sound gone). Another observation, systolic 108, 82 (abrupt diminution in sound); 65 (sound gone).

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.52	30.68°	30.76°		3.10	30.725°	30.865°	22.9°
2.56	30.62°	30.70°	23.1°	3.12	30.73°	30.875°	
2.58	30.675°	30.77°	22.9°	3.14	30.68°	30.85°	
3.00	30.68°	30.79°	22.9°	3.30	30.40°	30.58°	23.0°
3.02	30.695°	30.80°	22.9°	Cooling in 16 minutes of right calorimeter 0.28°, of left 0.27°. Volume of right foot in calorimeter 1,109 c.c. Volume of left foot in calorimeter 1,100 c.c.			
3.04	30.71°	30.83°	22.8°				
3.06	30.71°	30.83°	22.8°				
3.08	30.72°	30.855°	22.9°				

M. V. was examined for the first time the day following his admission to the hospital. His rectal temperature was 40.5° C. (104.9° F.), although he had had a full bath two hours before the examination. The flow was only 1.54 grams per 100 cubic centimeters of foot per minute for the right foot, and 1.50 grams for the left, with room temperature 22.2° C. On account of the hemorrhage mentioned in the protocol nine days after the first examination, it was not considered justifiable to carry out the original program, since, although the examination caused apparently very little disturbance to the patient, absolute rest was obviously enjoined in this case. Accordingly an interval of exactly four weeks elapsed between the first and the second examination of the blood flow. His rectal temperature at the second examination was 37.95° C. (100.3° F.). The foot flow was somewhat smaller than at the first examination (1.24 grams per 100 cubic centimeters per minute for the right, and 1.40 grams for the left, with room temperature 22.9°). Of course the amount of heat given off per minute to the calorimeter was much greater at the first examination than at the second on account of the higher blood temperature. This renders it necessary to state that it has not hitherto been feasible to determine by actual experiment in a fever case the relation between the rectal temperature and the temperature of the arterial blood at the ankle under the average conditions of our blood flow observations. It has been assumed in the calculations that the difference is 0.6° C., the same as for a normal person. It is, of course, possible that in fever the temperature of the blood as it passes from the heart to the wrist or ankle falls more than in health, since the excess of temperature of the skin over that of the surroundings is greater. Yet the arterial blood moves so rapidly and the large arteries are so well protected that it can scarcely be supposed that any considerable difference exists. It must be taken into account also that in the observations the limbs were covered as far as possible down to the calorimeters. It may therefore be confidently assumed that the calculations are not vitiated in any important degree by the want of direct experimental control of this factor.

The greatest number of observations were obtained on J. B.

J. B.—A laborer, aged 24 years, was admitted to the City Hospital on September 23, 1912, suffering from typhoid fever. He had been ill for three weeks with alternate attacks of diarrhea and constipation, and malaise. He was quite ill when brought to the hospital and was delirious on September 25. Spleen enlarged and tender. Pulse, low tension, small volume, and dicrotic. Maximum temperature September 25, 105.8° F.; temperature October 1 at 4 P.M. 104.0°; October 2 at noon 101.6°.

First Examination of Blood Flow.—October 2, 1912. The patient seemed quite ill when brought in on the ambulance and lay on his back perfectly still throughout the examination. Feet in bath at 1.18 P.M., and in calorimeters at 1.34½ P.M. His feet are small. 3,740 c.c. of water in each calorimeter. Pulse 108. Blood pressure, right arm, systolic 102 (palpation), 100 (stethoscope); 78 (sound gone).

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
1.32	31.03°	31.08°		1.51	31.32°	31.50°	Room temperature 24.35°.
1.37	30.93°	31.01°	24.3°	1.53	31.375°	31.58°	
1.39	30.96°	31.08°	24.4°	1.55	31.45°	31.67°	Feet out of calorimeters at 1.55.
1.41	31.00°	31.14°	24.4°				
1.43	31.06°	31.20°	24.3°	1.57	31.425°	31.65°	Room temperature 24.3°.
1.45	31.12°	31.28°		2.17	31.12°	31.35°	
1.47	31.19°	31.35°	24.4°				
1.49	31.26°	31.415°					

Cooling of right calorimeter in 20 minutes, 0.305°, of left 0.30°.
Volume of right foot 927 c.c., of left 945 c.c.
Rectal temperature 39.35°.

Second Examination.—October 8, 1912. The maximum temperature has continued high since the last examination. Temperature at 2.30 P.M. on October 7, 105.4° F., at 10 P.M. 105.0° F.; October 8 at 8 A.M. 101.0° F. The patient has had an ice cap on his head, and this was on during the examination. Feet in bath at 1.49 P.M., and in calorimeters at 2.04½ P.M. 3,740 c.c. of water in each calorimeter. Pulse 104. Blood pressure, right arm, systolic 109 (stethoscope and palpation); diastolic 80 (sound abruptly diminished), 65 (sound gone entirely).

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.	Notes.
	Right.	Left.			Right.	Left.		
2.02	30.74°	30.72°		2.20	30.295°	30.23°	22.4°	
2.06	30.59°	30.50°		2.22	30.26°	30.19°	22.4°	
2.08	30.52°	30.45°		2.24	30.23°	30.17°	22.1°	
2.10	30.47°	30.395°	21.4°	2.26	30.20°	30.145°		Feet out of calorimeters at 2.26.
2.12	30.43°	30.36°	21.6°					
2.14	30.39°	30.33°	21.9°					
2.16	30.36°	30.28°	22.1°	2.28	30.17°	30.10°		
2.18	30.32°	30.25°	22.2°	2.45	29.89°	29.82°	22.2°	

Cooling of right calorimeter 0.28° in 17 minutes, and of left 0.28°.
Volume of right foot 924 c.c., of left 900 c.c.
Rectal temperature at 3 P. M. 39.45° C.

The feet were somewhat blue when taken out of calorimeters.

Third Examination.—October 16, 1912. The patient still has the ice cap on. Temperature at noon on October 9, 99.8° F., at 4 P.M. 104.4°; October 10 at noon 101.4° F., at 8 P.M. 103.8°; October 11 at 6 P.M. 104.0° F. Since this time it has gradually fallen. On October 16 at 8 A.M. it was 99.4°, and at noon 101.0°. He says that he has no appetite. Blood pressure, right arm, systolic 104 (palpation), 105½ (stethoscope); 66 (sound gone). Pulse 106. Feet in bath at 1.54 P.M., in calorimeters at 2.08 P.M. 3,740 c.c. of water in each calorimeter.

Time.	Temperature of			Time.	Temperature of		
	Calorimeters.		Room.		Calorimeters.		Room.
	Right.	Left.			Right.	Left.	
2.05	31.29°	31.24°		2.32	30.62°	30.67°	23.1°
2.10	31.03°	31.04°	23.1°	2.34	30.60°	30.665°	22.9°
2.12	30.95°	31.01°	22.9°	2.36	30.58°	30.65°	
2.14	30.91°	30.95°	22.8°	2.38	30.55°	30.61°	23.1°
2.16	30.86°	30.91°	22.7°	2.40	30.54°	30.61°	23.1°
2.18	30.82°	30.87°	22.6°	2.42	30.52°	30.60°	22.9°
2.20	30.79°	30.84°	22.8°	2.44	30.51°	30.58°	22.9°
2.22	30.76°	30.81°		2.46	30.50°	30.575°	23.0°
2.24	30.72°	30.775°	23.2°	2.48	30.50°	30.57°	23.1°
2.26	30.695°	30.755°	23.4°	2.50	30.495°	30.565°	23.0°
2.28	30.67°	30.73°	23.6°	2.52	30.49°	30.56°	
2.30	30.65°	30.71°	23.4°	2.54	30.46°	30.50°	
				3.09	30.23°	30.27°	23.05°

Cooling of calorimeters in 15 minutes, right 0.23°, left 0.23°. Feet out of calorimeters at 2.52.

Volume of right foot 834 c.c., of left 837 c.c. Water equivalent of calorimeters with contents, right 4,444 c.c., left 4,446 c.c. Rectal temperature 40.2° C.

Fourth Examination.—November 1, 1912. The patient's temperature is now about normal and he feels much better. The maximum and minimum temperatures were as follows:

October 16, 105.0° and 99.4° F.	October 17, 103.4° and 100.4° F.
October 18, 104.0° and 100.4° F.	October 19, 104.0° and 100.0° F.

Since October 19 the temperature has gradually fallen. On October 31 the maximum was 99.0° F. and the minimum 98.0° F.

Pulse (lying on back on the ambulance) 87. Feet in bath at 2.07 P.M., and in calorimeters at 2.21 P.M. 2,775 c.c. of water in each calorimeter. The calorimeters had been reduced in size since the last examination. The soles of the feet rest on the footrest as before. Consequently a smaller portion of the leg is now inside the calorimeter. Blood pressure, left arm, systolic 108, 84 (sound gone).

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of Calorimeters.		Room.	Notes.
	Right.	Left.			Right.	Left.		
2.23	30.56°	30.65°		2.45	30.24°	30.295°	22.0°	Feet out of calorimeters at 3.02.
2.25	30.495°	30.58°	21.4°	2.47	30.25°	30.30°	22.1°	
2.27	30.41°	30.52°	21.4°	2.49	30.265°	30.31°	22.15°	
2.29	30.37°	30.46°	21.5°	2.51	30.28°	30.32°	22.2°	
2.31	30.32°	30.43°	21.7°	2.53	30.305°	30.33°	22.25°	
2.33	30.29°	30.38°	21.8°	2.55	30.35°	30.345°	22.2°	
2.35	30.275°	30.355°	21.9°	2.57	30.365°	30.355°	22.3°	
2.37	30.26°	30.34°	21.9°	2.59	30.385°	30.365°	22.4°	
2.39	30.25°	30.33°	22.0°	3.01	30.395°	30.38°	22.4°	
2.41	30.235°	30.31°	22.0°	3.03½	30.34°	30.335°		
2.43	30.235°	30.30°	22.0°	3.25	29.97°	29.955°		

Cooling of right calorimeter in 21½ minutes 0.37°, of left 0.38°.
Volume of right foot in calorimeter 780 c.c., of left 740 c.c.
Water equivalent of right calorimeter and contents 3,560 c.c., of left 3,530 c.c.

The first examination of J. B. was made on October 2, 1912, ten days after his admission, more than four weeks from the beginning of his illness, and a week after the maximum temperature (105.8° F.) had been reached. The rectal temperature after the examination was 39.35° C. (102.83° F.). The flow in the feet was the largest observed in this series of fever cases and the only flow which fell within the normal range (3.36 grams per 100 cubic centimeters per minute for the right foot, and 3.82 grams for the left, with room temperature 24.4° C.).

Six days later, his temperature having meanwhile mounted again and continued high, the foot flow was found to be much smaller (table II, footnote), and eight days later, on October 16, the rectal temperature being now 40.2° C. (104.36° F.) the flow was only 0.80 grams for the right foot and 0.74 grams for the left. The clinical records show great variations in temperature since the first examination and he continued to present disturbing symptoms, cardiac and otherwise.

The last examination was made on November 1, 1912, almost seven weeks after admission to the hospital and ten weeks after the beginning of the illness, his temperature being now for the first time fairly stable and at a normal level. The flow was 2.08 grams per 100 cubic centimeters per minute for the right foot and 1.89 grams for the left. In view of the fact that the room temperature was

more than 2° C. lower on this than on the first occasion, the results indicate that at the first examination the conditions that determine the rate of flow in the feet were more similar to the conditions at the last examination than to those at the two intermediate examinations, in spite of the great difference of body temperature. The most natural explanation is that both at the first and the last examination in this rather protracted case the intoxication was subsiding and the cutaneous vasoconstriction was disappearing. After the first examination, however, a relapse seems to have occurred, the patient's progress being interrupted by complicating incidents (continued ulceration, etc.), the cutaneous vasoconstriction increased, and the temperature ran up again almost, if not quite, to the maximum reached before. In the absence of simultaneous measurements of the hand flow it is impossible to know whether intoxication of the heart contributed to the smallness of the foot flows in the intermediate period, although from the heart symptoms this seems probable. The progressive diminution in the volume of the feet in the first three examinations is a point which may be noted since it affords a quantitative expression (for a particular part) of the increasing emaciation which was very evident in the body as a whole. The foot volume at the fourth examination is not comparable with the others as the reduced calorimeters were then used for the first time.

In F. B., a case of pneumonia, the flow at the first examination, on the day of his admission to the hospital and a fortnight from the beginning of his illness, was 2.07 grams per 100 cubic centimeters of foot per minute for the right foot, and 2.30 grams for the left, with room temperature 23.0° C.,—flows not perhaps markedly deficient for his age, but certainly not above the normal. Six days later the flow was less (1.36 grams for the right, and 1.12 grams for the left foot) with a slightly lower room temperature, but practically the same rectal temperature. Here again in the absence of observations on the hands it cannot be stated what share the weakening of the heart might have had in the diminution of the flow. That it was weakening at this time is probable, as he died four and a half days later, apparently from a general intoxication.

F. B.—A man, aged 53½ years, a cook by occupation, was admitted to the City Hospital on December 5, 1912, with lobar pneumonia, the lower lobe of the left lung being consolidated. His illness began two weeks before admission, with pain in the left side, chills, and fever. Cough and shortness of breath developed shortly after. But he did not go to bed, and indeed he walked into the hospital. The maximum and minimum temperatures were:

December 6, 103.8° and 103.0° F. December 7, 104.0° and 102.6° F.
December 8, 103.8° and 102.6° F. December 9, 103.2° and 101.0° F.
December 10, 103.0° and 101.2° F.

The leucocyte count was as follows:

December 6, 8,600 December 7, 12,400
December 8, 18,400 December 9, 20,400

On December 5 the blood flow in the feet was measured while the patient lay on his back. Pulse 92. Feet in bath at 2.04 P.M., and in calorimeters at 2.16 P.M. 2,775 c.c. of water in each calorimeter. The calorimeters of reduced capacity were used.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
2.14	31.49°	31.65°	23.7°	2.28	31.67°	31.87°	Room temperature 22.7°.
2.18	31.46°	31.63°	23.8°	2.30	31.71°	31.925°	
2.20	31.50°	31.69°	23.5°	2.32	31.77°	31.97°	Feet out of calorimeters
2.22	31.54°	31.755°	22.9°				at 2.32.
2.24	31.59°	31.79°	22.9°	2.34	31.765°	31.955°	
2.26	31.62°	31.84°	22.8°	2.49	31.50°	31.695°	

Cooling of right calorimeter 0.265° in 15 minutes, of left 0.265°.
Volume of right foot 1,081 c.c., of left 1,044 c.c. Rectal temperature 39.4°.

Blood pressure, left arm, systolic 104 (stethoscope), 103 (palpation); 84 (sudden decrease in sound), 58 (sound gone).

At the second examination of F. B. on December 11, 1912, the feet were put into the bath at 1.55 P.M., and into the calorimeters at 2.11½ P.M. Pulse (lying on back, as in the previous examination) 112. 2,775 c.c. of water in each calorimeter, the calorimeters of reduced capacity being used.

Time.	Temperature of Calorimeters.		Room.	Time.	Temperature of calorimeters.		Notes.
	Right.	Left.			Right.	Left.	
2.09	31.25°	31.17°		2.29	31.28°	31.13°	
2.14	31.15°	31.07°		2.31	31.295°	31.135°	Room temperature 22.1°.
2.17	31.17°	31.08°	21.0°	2.33	31.305°	31.14°	Room temperature 22.1°.
2.19	31.19°	31.085°	21.3°	2.35	31.325°	31.14°	Feet out of calorimeters
2.21	31.205°	31.095°	21.7°				at 2.35.
2.23	31.22°	31.11°	21.8°	2.37½	31.29°	31.09°	
2.25	31.25°	31.12°	21.8°	2.50½	31.06°	30.86°	
2.27	31.265°	31.13°	21.9°				

Cooling of right calorimeter 0.23° in 13 minutes, of left 0.23°.
Volume of right foot 1,072 c.c., of left 1,023 c.c. Rectal temperature 39.3°.

Water equivalent of calorimeters and contents, right 3,779 c.c., left 3,742 c.c. The patient died on December 15, the whole left lung being consolidated before death. The systolic blood pressure was 96 on December 12, 80 on December 13, and 90 on December 14, all the observations being taken in the afternoon.

TABLE I.

	Date.	Heat given off per min. in small calories.		Heat given off per 100 c.c. of foot per min.		Heat given off per gm. of blood.		Rectal temperature.
		Right.	Left.	Right.	Left.	Right.	Left.	
	Normal Individuals.							
M.C.	May 2, 1911	251.6	—	20.6	—	5.77	—	37.4°
	May 18, 1911	211.6	—	17.4	—	4.93	—	37.5°
	June 17, 1911	332.3	278.8	28.4	25.6	5.76	6.06	37.6°
	Mar. 26, 1913	290.2	290.2	23.5	24.2	4.60	4.64	37.1°
C.B.	May 2, 1911	262.1	—	26.7	—	5.65	—	36.9°
J.R.	Mar. 25, 1913	351.7	327.1	34.8	34.1	4.37	4.49	37.0°
N.R.	Apr. 8, 1913	83.8	70.6	17.0	14.7	5.05	5.14	37.2°
	Fever Patients.							
F.B.	Dec. 5, 1912	145.0	151.5	13.4	14.5	6.41	6.29	39.4°
	Dec. 11, 1912	98.0	78.4	9.1	7.6	6.69	6.83	39.3°
J.B.	Oct. 2, 1912	211.3	240.0	22.7	25.3	6.78	6.63	39.35°
	Oct. 16, 1912	54.7	50.2	6.5	6.0	8.18	8.12	40.2°
	Nov. 1, 1912	95.7	81.6	12.2	11.0	5.87	5.81	37.45°
M.V.	Oct. 8, 1912	163.0	157.9	12.3	12.2	8.00	8.13	40.5°
	Nov. 5, 1912	81.8	90.8	7.3	8.2	5.91	5.87	37.95°
F.S.	Oct. 2, 1912	94.7	90.2	7.3	7.1	4.97	5.13	37.9°
F.D.	Oct. 1, 1912	56.8	49.1	4.1	4.3	5.12	5.19	37.2°

TABLE II.

	Date.	Age in yrs.	Pulse rate.	Blood pressure.	Temperature of Room.	Temperature of Arterial blood.	Temperature of Calorimeters. Right.	Temperature of Calorimeters. Left.	Volume of part in c.c. Right.	Volume of part in c.c. Left.	Heat given off in small calories. Right.	Heat given off in small calories. Left.	Heat given off in small calories. In min.	Blood flow per min. in gm. Right.	Blood flow per min. in gm. Left.	Flow per 100 c.c. of part per min. Right.	Flow per 100 c.c. of part per min. Left.	Notes.
								Normal Individuals.										
M. C. Hands.	May 4, 1911	22	90		23.3°	36.7°	30.04°	30.03°	486	470	3,380	2,950	9	62.48	54.60	12.86	11.61	Left forearm bandaged before experiment and bandage removed.
	May 24, 1911		87		27.0°	36.8°	30.91°	30.91°	470	457	3,228	2,976	7	86.99	80.20	18.51	17.55	
	Nov. 2, 1911		76		22.2°	36.5°	29.78°	29.77°	465	435	884	878	3	48.72	48.31	10.48	11.10	Cuff on right arm, but no pressure.
	Nov. 3, 1911		88	115	25.1°	36.75°	30.01°	30.04°	493	459	1,815	1,627	5	59.84	53.88	12.14	11.74	Cuff on right arm, but no pressure.
	Nov. 9, 1911		87	115	24.1°	36.7°	30.84°	30.88°	465	465	2,357	2,046	7	63.84	55.80	13.73	12.0	12.54 allowing for swelling of left hand.
	Nov. 15, 1911		—	113, 85	25.2°	36.8°	30.79°	30.83°	480	461	2,932	2,737	8	67.53	63.68	14.07	13.81	
	Nov. 27, 1911		100	124	21.4°	36.9°	29.92°	29.87°	477	455	1,617	1,356	7	36.77	30.57	7.71	6.72	
	Dec. 11, 1911		74	122, (92), 82	24.5°	36.6°	31.18°	31.12°	450	444	2,315	2,225	$8\frac{1}{2}$	55.83	53.17	12.41	11.98	
	Mar. 26, 1913	24	97		24.5°	36.6°	31.53°	31.61°	505	485	2,712	2,612	8	74.29	73.28	14.71	15.10	
					24.5°		32.27°				3,289		14	60.24		11.93		Left hand in cold water.
					24.6°		32.73°				507		3	48.52		9.60		Left hand in warm water.
					24.5°		33.10°				1,889		8	74.96		14.84		Left hand still in warm water.
M. C. Feet.	May 2, 1911	22			25.25°	36.8°	30.38°		1,220		1,510		6	43.55		3.57		Foot at rest.
					25.4°		30.55°				1,253		5	44.55		3.65		Working foot.
					25.2°		30.71°				1,437		6	43.69		3.58		Foot at rest.
	May 18, 1911		76		26.4°	36.9°	31.42°		1,210		2,116		10	42.90		3.54		
	June 17, 1911		84		21.8°	37.0°	30.60°	30.26°	1,170	1,085	3,988	3,346	12	57.69	45.97	4.93	4.24	
	Mar. 26, 1913	24	92		24.5°	36.5°	31.38°	31.34°	1,232	1,195	4,064	4,064	14	62.99	62.50	5.11	5.23	Lying on back.
			98		24.5°		32.06°	32.03°			3,119	3,212	16	48.78	49.90	3.96	4.17	Sitting.
					24.6°			32.46°				1,355	10		37.26		3.12	Right foot in cold water.
					24.6°			32.76°				1,664	8		61.79		5.17	Right foot still in cold water.
					24.6°			32.84°				930	6		47.05		3.93	Right foot in warm water.
					24.6°			33.16°				2,747	10		91.38		7.64	Right foot still in warm water.

TABLE II.—*Continued.*

	Date.	Age in yrs.	Pulse rate.	Blood pressure.	Temperature of Room.	Temperature of Arterial blood.	Calorimeters. Right.	Calorimeters. Left.	Volume of part in c.c. Right.	Volume of part in c.c. Left.	Heat given off in small calories. Right.	Heat given off in small calories. Left.	Heat given off in small calories. In min.	Blood flow per min. in gm. Right.	Blood flow per min. in gm. Left.	Flow per 100 c.c. of part per min. Right.	Flow per 100 c.c. of part per min. Left.	Notes.
Normal Individuals.																		
N. R.	Apr. 8, 1913	8¾	84		24.0°	36.7°	30.97°	31.10°	203	181	1,292	1,226	15	16.70	16.21	8.22	8.95	Hands.
					24.0°	36.6°	30.98°	30.88°	491	480	1,509	1,272	18	16.57	13.72	3.37	2.86	Feet.
Fever Patients.																		
F. B.	Dec. 5, 1912	53½	92	104, 58	23.0°	38.8°	31.62°	31.80°	1,081	1,044	2,176	2,273	15	22.45	24.05	2.07	2.30	Pneumonia. Feet.
	Dec. 11, 1912		112		22.0°	38.7°	31.24°	31.11°	1,072	1,023	2,059	1,646	21	14.63	11.47	1.36	1.12	
J. B.	Oct. 2, 1912	24	108	100, 78	24.4°	38.75°	31.21°	31.38°	927	945	3,382	3,840	16	31.15	36.18	3.36	3.82	Typhoid. Feet.
	Oct. 8, 1912		104	109, 65	22.2°	38.85°	30.36°	30.35°	924	900	—	—	—	—	—	—	—[2]	
	Oct. 16, 1912		106	104, 66	23.0°	39.6°	30.50°	30.58°	834	837	547	502	10	6.68	6.18	0.80	0.74	
	Nov. 1, 1912		87	108, 84	22.2°	36.85°	30.32°	30.34°	780	740	1,531	1,306	16	16.28	14.04	2.08	1.89	
M. V.	Oct. 8, 1912	29	108	118, 89	22.2°	39.9°	30.97°	30.86°	1,322	1,294	2,292	2,211	14	20.37	19.41	1.54	1.50	Typhoid. Feet.
	Nov. 5, 1912		80	107, (92) 65	22.9°	37.35°	30.80°	30.82°	1,109	1,100	1,145	1,272	14	13.82	15.45	1.24	1.40	
F. S.	Oct. 2, 1912	28	72	100, (77), 58	24.1°	37.3°	31.77°	31.59°	1,295	1,258	2,180	2,076	23	19.04	17.56	1.47	1.39	Typhoid. Feet.
F. D.	Oct. 1, 1912	26	81	108, 84	22.9°	36.7°	31.91°	31.88°	425	423	1,511	1,426	9	38.94	36.50	9.16	8.63	Typhoid (convalescent). Hands, feet.
					22.45°	36.6°	30.90°	30.82°	1,135	1,117	910	787	16	11.08	9.45	0.98	0.85	
J. McD.	May 16, 1912	26	80		23.2°	36.7°	31.28°	31.52°	511	505	3,118	3,568	18	35.51	42.52	6.95	8.42	Typhoid (convalescent). Hands.
					23.2°			31.99°				654	5		30.83		6.10	Right hand in cold water.
					23.1°			32.14°				833	5		40.59		8.04	Right hand still in cold water.
					23.3°			32.34°				1,242	10		31.65		6.26	Right hand in warm water.

[2] The flow was so small that even after the feet had been in the calorimeters for 21½ minutes the loss of heat by the calorimeters to the room was not yet balanced. The feet were withdrawn before usable readings were obtained. From the readings obtained it is practically certain that the foot flow was not greater than on October 16.

	Date.	Age in yrs.	Pulse rate.	Blood pressure.	Temperature of				Volume of part in c.c.		Heat given off in small calories.			Blood flow per min. in gm.		Flow per 100 c.c. of part per min.		Notes.
					Room.	Arterial blood.	Calorimeters. Right.	Calorimeters. Left.	Right.	Left.	Right.	Left.	In min.	R g ht.	Le t.	Right.	Left.	
Normal Individuals.																		
C. B. Hands.	May 22, 1911	22	80		28.9°	36.6°	31.15°	31.21°	415	410	3,975	4,176	16	50.65	53.83	12.20	13.13	
					29.0°		31.76°				497		4	28.52		6.95		Left hand in cold water.
					29.0°		31.96°				1,028	.	6	41.03		9.89		Left hand still in cold water.
					29.1°		32.36°				2,022		11	48.17		11.60		Left hand in warm water.
	Nov. 17, 1911		106	103	24.9°	36.5°	30.15°	30.29°	430	424	2,338	2,712	8½	49.22	57.09	11.45[1]	13.46[1]	
	Nov. 20, 1911			113	24.8°	36.45°	30.68°	30.66°	410	405	1,369	1,334	10	26.36	25.55	6.43[1]	6.30[1]	
	Nov. 21, 1911		104	115	24.2°	36.75°	31.28°	31.36°	410	405	2,096	2,060	9	47.30	46.83	11.54[1]	11.42[1]	
	Nov. 24, 1911		80	116	23.5°	36.6°	30.48°	30.40°	400	385	785	953	6	23.56	28.46	5.89[1]	7.39[1]	Hands felt cold though not to the subject.
C. B. Foot.	May 2, 1911		72		26.3°	36.3°	30.02°		980		1,573		6	46.38		4.73		Foot at rest.
					26.3°		30.20°				1,145		4	52.14		5.32		Working foot.
					26.0°		30.36°				1,216		4	56.82		5.79		Foot at rest.
J. R. Hands.	Mar. 22, 1913	20	52		24.0°	36.0°	30.90°	30.88°	397	354	2,388	1,537	13	40.02	25.66	10.08	7.25	
					24.2°		31.30°				1,092		13	19.85		5.00		Left hand in cold water.
					23.95°		31.42°				113		2	13.70		3.45		Left hand in warm water.
					23.9°		31.49°				409		3	33.59		8.46		Left hand still in warm water.
					23.95°		31.72°				1,610		7	59.70		15.04		Left hand still in warm water.
	Mar. 25, 1913		62	101, 60	24.8°	36.5°	31.62°	31.55°	393	376	3,206	2,217	15	48.66	33.17	12.38	8.82	
					24.4°		32.11°				460		5	23.28		5.92		Left hand in cold water.
					24.4°		32.25°				802		5	41.93		10.67		Left hand still in cold water.
					24.4°		32.37°				347		3	31.11		7.91		Left hand in warm water.
					24.5°		32.61°				1,699		7	69.32		17.64		Left hand still in warm water.
J. R. Feet.	Mar. 25, 1913		76		25.1°	36.4°	31.54°	31.40°	1,008	959	4,925	4,580	14	80.42	72.70	7.98	7.58	Lying on back.
					24.6°		32.60°	32.34°			4,869	4,359	18	79.09	66.27	7.84	6.90	Sitting.
					24.5°			32.87°				1,440	10		45.32		4.72	Right foot in cold water.
					24.45°			33.08°				1,405	8		58.77		6.12	Right foot in warm water.
					24.45°			33.35°				1,753	8		79.82		8.27	Right foot still in warm water.

[1] The cuffs of the sphygmomanometer were on the arms, but were not inflated.

CONCLUSIONS.

1. In the cases of fever investigated the flow in the feet never exceeded the normal flow and was usually much below the normal

2. In explanation of the relatively small foot flow in the fever cases it is suggested that the vasoconstrictor mechanism of the peripheral parts, especially of the skin, is abnormally excited, and some direct evidence that this is the case is brought forward.

3. The significance of this hypersensitiveness, or at least increased action, of the cutaneous vasoconstrictor mechanism is assumed to be that the peripheral vasoconstriction is a compensatory arrangement which secures for the organs mainly suffering from the infective process an increased flow of blood.

4. On this hypothesis the rise of temperature is, chiefly at least, secondary, inevitably following the vasoconstriction, provided that the metabolism is, upon the whole, not diminished.

5. Accordingly the rational treatment of hyperpyrexia, or of pyrexia if it is considered necessary to treat it, is to abstract heat by a process which will not diminish and may even increase the cutaneous vasoconstriction. This condition is exactly fulfilled by the cold bath, at least as regards its initial effect. Other so called tonic effects of the cold bath are not considered here.

Antipyretic drugs which act by dilating the cutaneous vessels would seem to be inferior in this regard. They diminish the temperature, it is true, but at the cost of defeating the beneficial redistribution of the blood which it is the function of the peripheral vasoconstriction to insure.

6. It is obvious that for the elimination of a given quantity of heat from the skin by radiation and conduction[3] in fever, a smaller cutaneous blood flow will suffice than with normal body temperature, since the elimination of heat per gram of blood passing through the surface must be greater in fever owing to the greater difference of temperature between the surface of the body and its surroundings.

I am much indebted to the staff of the City Hospital for their coöperation which has rendered this investigation possible.

[3] Loss of heat by sweat is not considered since the parts investigated could not lose heat in this way under the conditions of the observations.

A STUDY OF THE BLOOD IN RATS RECOVERED FROM IMPLANTED SARCOMA.*

By RICHARD WEIL, M.D.

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The studies reported in this paper were made in the attempt to determine whether the factor of immunity can be demonstrated in the blood of animals which have recovered from implanted tumors. Two series of experiments were carried out, each from a different standpoint.

THE PASSIVE TRANSFERENCE OF IMMUNITY.

In the first series large amounts of immune blood plasma were injected into animals with growing tumors, and also into normal animals subsequently implanted with tumors, in order to determine the effect upon the tumor process. Similar experiments were reported in 1905 by Gaylord, Clowes, and Baeslack.[1] Their results,

* Received for publication, May 17, 1913.

[1] Gaylord, H., Clowes, G. H. A., and Baeslack, F. W., *Med. News*, 1905, lxxxvi, 91. They state: "The blood-serum of mice which had recovered spontaneously from tumors possessed a power, when injected into mice infected with growing tumors, of inhibiting the growth of large tumors and causing the retrogression of smaller tumors, leaving the animal possessed of an immunity which prevents recurrence of the growth. The degree of immunity in the mice thus far tested varies within considerable limits, the most marked illustration of its activity being found in one mouse whose blood-serum injected in a single dose of .2 c.c. caused the rapid retrogression and entire disappearance of two tumors in one animal and one in another, all of which were as large as peas, in the space of three days. The same serum injected into a mouse with a tumor the size of a small cherry (about two grams) caused a noticeable reduction in size of the tumor, which remained stationary for ten days, when an operation for the removal of a portion of it resulted in a return of activity to the growth. The latter part of this experiment will be dealt with in our final publication.

"All of these experiments were controlled with mice inoculated at the same time, the tumors of which were smaller than those of the mice treated with the

however, have not been confirmed by any extensive series of experiments. Lewin,[2] indeed, obtained some cures in both rats and mice by this method, using the serum of immune animals approximately fourteen days after a negative inoculation in the latter. He is under the impression that the "serum of such animals does actually contain immune substances."

Before proceeding to my experiments it may be well to state that no method hitherto employed has effectively demonstrated the presence of immune substances in the serum. In this general statement are comprehended not only the results of a large number of test-tube determinations, but also the results obtained by Lambert and Hanes by means of artificial cultivation. Although individual observers have occasionally published data that seem to indicate the presence of specific reaction products in the blood of animals either suffering from or recovered from tumors, there has never been a general confirmation of these views. The results obtained by the biological method, employed by Gaylord and his co-workers, and partially confirmed by Lewin, would therefore be valuable, as indicating the existence of immune substances, even though they are not demonstrable by the present methods of test-tube analysis.

In the following experiments a rat sarcoma was employed,[3] which under favorable circumstances has a record of 80 to 100 per cent. of takes. After an average period of growth ranging from

immune serum. These control mice received doses of normal mouse serum equal in volume to the doses of immune serum referred to above. In every case the tumors in the control mice developed rapidly and led in the course of three or four weeks to the death of the animal. In spite of the fact that the control tumors were invariably smaller than those used for the immune serum at the commencement of each experiment, in the course of a week or ten days the control tumors were found to be larger, and up to the date of making this announcement, while several control mice have died of their tumors, not a single mouse treated with immune serum has so far succumbed. In those cases in which the tumor was too large or the immune serum too weak to effect a cure, the marked retardation in the development of the tumor was always associated with a diminution in the cachectic symptoms invariably exhibited by the tumor mice in the last stages."

[2] Lewin, C., *Berl. klin. Wchnschr.*, 1913, 1, 153.

[3] The sarcoma was obtained many years ago from the Cancer Laboratory, in Buffalo, and has since then been under constant observation in our laboratory.

two to four weeks, a considerable number of the takes begin to retrogress, and either completely disappear or are reduced to small and indolent masses. The exact percentage of retrogression varies considerably in different series, and does not seem to depend upon any demonstrable factor. In every case, therefore, it is necessary to control an experimental series by an observation of at least an equal number of controls, both sets of animals having been inoculated at the same time, from the same tumor, and by the same method. The immune serum was invariably obtained by the following procedure. A large number of inoculated rats were constantly kept in stock. From this group a number of animals were selected whose tumors had undergone total retrogression within a period not greater than ten days. The animals were bled to death by section of the thorax, and the blood was defibrinated and centrifuged. Spontaneously recovered animals were used in preference to those that had not taken the inoculation (nullers), for the reason that the former were found, over a long series of experiments, to be almost uniformly refractory to a second inoculation, which is not the case with the latter. Recovered animals are, therefore, distinctly immune, whereas nullers are not necessarily so.

The method of immunization by previous injection of killed tumor or other tissue was discarded, in view of the fact that animals so treated become immune only in an uncertain, often small, proportion of the cases.

In the first series of animals that was studied in this connection, twenty-four young white rats were included. These were divided into two equal lots of twelve each. Of the first lot, six rats received an intravenous injection into the jugular vein of one cubic centimeter of plasma, obtained from recovered rats. One cubic centimeter represents the average amount of plasma which it was possible to obtain from one rat. The other six received two cubic centimeters of the same plasma intraperitoneally. On the day following this injection, all the animals received a subcutaneous inoculation, by the trocar method, of rat sarcoma. After ten days the animals were examined. Of the treated series, nine, and of the untreated series, ten showed growths. It was not possible at this time, in view of the minute size of the tumors, to determine any

difference quantitatively. On the eighteenth day the animals were again examined. Of the treated series ten remained alive, of which again nine showed tumors. Of the untreated series the total number remained alive, and ten showed tumors. There were no quantitative differences discernible in the average growth of the tumors. On the thirtieth day there had been retrogression in all except four of the treated series, and in all except three of the untreated series. At this point the observation of both series was discontinued. The conclusion was drawn that the preliminary inoculation of immune serum does not protect rats against subsequent implantation of tumors, and does not noticeably influence the rate of growth, nor the percentage of retrogressions in such tumors.

In another series of animals the conditions of the experiment were varied by making, in addition to the preliminary treatment with immune serum on the day preceding the tumor inoculation, a subsequent injection of serum five days thereafter. It seemed possible that the second injection, being made at a critical period in the history of the graft, might conceivably exercise a more potent influence than the earlier injection. It does not seem necessary to give the details of this second experiment. In brief, fourteen controls were used, and eight treated animals. The percentage of takes and of recoveries in the two series differed by so little that it was evident that the treatment had produced no therapeutic effect.

In spite of the fact that it had not proved possible materially to influence the process of tumor implantation by means of the injection of immune serum, it seemed possible that the rate of tumor growth, or of retrogression, might conceivably show the influence of repeated treatments of this nature. Theoretically, it is possible that immune substances may be ineffective in preventing successful implantation, whereas they may be of importance in controlling growth.

In order to determine this point rats were selected whose tumors had shown progressive growth, and had reached a size measuring not less than 1.5 centimeters in diameter. These rats, in favorable series, offer a fair prospect of continued growth without retrogression. It is necessary, however, to correct the results obtained

in a treated series, by the observation of a considerable number of exact controls planted from the same tumor at the same time.

Fourteen animals were selected for treatment. All the animals had been inoculated twenty-five days previously. There were 100 per cent. of takes, and on the day on which observation began all of the tumors were actively growing and showed no ulceration. The rate of growth of each tumor was plotted by a second observation made ten days later, or on the thirty-fifth day after inoculation. Upon that day each of the animals received an injection of immune plasma. In five, 0.5 of a cubic centimeter was injected intravenously. In the other nine, one cubic centimeter was injected intravenously. In all the animals three further injections, at intervals of two days, each of one cubic centimeter of plasma from immune rats, were made intraperitoneally. The size of the tumors was again plotted seven days after the beginning of treatment, and again fourteen days thereafter. All but two of the animals survived the treatment and were in good condition. In all the twelve that survived, except two, there had been continuous striking increase in the growth of the tumor, which at this stage is unusually active. In the two exceptions there had been retrogression which, in one, had been almost complete. Comparison with the controls brought out the fact that the percentage of retrogressions in this series was greater by 12 per cent. than in the treated animals. In the rest of the series the rate of growth was approximately the same as in the ten of the first series described above. It does not seem justifiable to infer that the injection of the immune serum improved the growth in the first series. The treatment, however, seems to have been entirely ineffective in controlling tumor growth.

The conclusion is therefore drawn that the repeated injection of plasma, derived from immune rats, failed to influence favorably the growth of a certain strain of rat sarcoma. Therefore, as far as the results of this series of experiments justify generalization, one may conclude that the blood of recovered rats does not carry the factor of immunity present in these animals.

PASSIVE SENSITIZATION.

One of the best methods at present available for the determination of immune substances in serum is that afforded by passive

anaphylaxis. If a rabbit is immunized by repeated injections of horse serum, the presence of immune substances may be demonstrated in the serum of the rabbit by the following method: normal guinea pigs are given an intraperitoneal injection of the rabbit serum, and on the next day an intravenous injection of horse serum. If the rabbit was immunized the guinea pigs become anaphylactic. Thus, guinea pigs treated with 0.05 of a cubic centimeter of immune rabbit serum may be killed by 0.01 of a cubic centimeter of horse serum.

It seemed possible that by the use of this method immune substances might be revealed in the blood of rats immune to tumor implantation, even though other methods, either biological or serological, had failed to reveal such substances. In order to determine this fact the following experiment was performed.

A large number of rats that had recently recovered from implanted tumors were bled to death on the same day and the plasma thus obtained was pooled. Quantities of this plasma, ranging in amount from 0.5 to four cubic centimeters, were injected intraperitoneally into six guinea pigs. Two days later each of these guinea pigs received an intravenous injection of an extract of the actively growing tumor, freshly removed, in normal salt solution; in each instance, one cubic centimeter of the extract was injected. None of the guinea pigs manifested more than slight discomfort. The fatal dose of this extract was not determined. Two and a half cubic centimeters, injected intravenously into normal guinea pigs, of approximately the same size as those that had received the preliminary intraperitoneal injection, produced marked prostration and weakness. All the controls, however, recovered.

In view of the fact that 0.005 of a cubic centimeter, or less, of a dose of foreign proteid, such as is ordinarily non-toxic in doses of 200 or 300 times that amount, is apt to produce fatal results in a sensitized guinea pig, it seems safe to conclude that the above experiment proves that guinea pigs cannot be sensitized by means of the blood of recovered rats.

In the second series of experiments, recovered rats received a series of subcutaneous inoculations of tumor extract, in order, if possible, to heighten their immunity. The animals were then bled.

and the plasma was injected into guinea pigs, as in the previous series. In this series again, however, there was complete failure to demonstrate sensitization.

The conclusion is therefore drawn that it is impossible, by means of the passive sensitization of guinea pigs, to demonstrate the presence of immune substances in the blood of rats recovered from an implanted sarcoma, or in the blood of rats recovered from this tumor and subsequently injected therewith.

THE MECHANISMS OF METASTASIS FORMATION IN EXPERIMENTAL CANCER.*

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PLATES 25 AND 26.

The term metastasis was originally used to indicate the transportation of any substance, whether metal or coal dust, normal cells (placenta, bone marrow), bacteria, or cancer cells from one part of the organism to another. von Recklinghausen (1) was the first to restrict the meaning of the term and used it only in connection with the transportation of pathological substances. More recently the word metastasis has been used only in connection with the secondary tumor nodules which occur so frequently in malignant growths. This restricted use of the term was caused by the generally recognized fundamental difference which exists between a multiple granuloma of an infectious disease and a true metastatic growth of a malignant tumor. While in the former only the bacteria or the noxious agents are transported into the distant parts of the organism where the granuloma is formed from the local cells, a metastatic tumor growth originates in every case through the proliferation of a group of cancer cells detached and transported from the primary tumor.

The mechanism of the formation of metastasis in malignant growths consists of the following phases: (1) the detachment of a group of cancer cells from the primary tumor; (2) the transportation of this group of cells through the blood or lymph channels into distant parts of the organism; and (3) the proliferation of these cells in the new location and the consequent formation of metastatic tumors. It is well known that both the frequency of the

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occurrence of metastases and the localization of the metastatic growths varies widely in different malignant tumors.

Most pathologists (von Recklinghausen (2), Lubarsch (3), and Schmidt (4)) consider the difference in the channels of transportation to be the main reason for the difference in the frequency and localization of the metastatic tumors. The causes are purely mechanical, namely, the differences in the morphological structure of the organs, the relative size of the cancer cells, the capillaries of a certain organ, and so on.

More recently Albrecht (5) and Ehrlich (6) advanced the hypothesis that the success of the development of a metastatic tumor is due to the chemical metabolic conditions of the entire organism. Their idea is that the growth of the secondary tumor depends upon the character of the primary growth. If the latter is very malignant and reaches a large size, then it uses up all the specific food required by the multiplying cancer cells, and none of it remains for the cells of the secondary growth. On the other hand, when the primary tumor is growing slowly, a sufficient amount of the specific food is left for the development of the metastatic tumor. Recent investigations of the writer (7) indicate that neither the purely mechanical conceptions nor the athreptic theory of Ehrlich are adequate to explain all the phenomena of the formation of metastasis.

The investigations were conducted mainly on two inoculable tumors of the white rat,—a spindle cell sarcoma which was studied originally by Jensen, and an adenocarcinoma which was described by Flexner and Jobling (8). The sarcoma is very malignant, grows to a large size, and apparently, in accordance with Ehrlich's theory, does not form any metastases after a subcutaneous inoculation. When an emulsion of this tumor was injected intravenously into a healthy animal no tumor growth formed anywhere. In these experiments lack of specific food could not play any part since the injected animals had no primary tumor. On the other hand, when the same tumor was inoculated into a parenchymatous organ, the spleen or the liver, metastases formed in a certain number of cases.

The second tumor, the Flexner-Jobling adenocarcinoma of the white rat, induces the formation of metastases when inoculated subcutaneously. An intravenous injection of an emulsion of this tumor induced the formation of metastatic nodules in parenchymatous organs.

In the two series of experiments the conditions within the host, both as regards the mechanical structure of the organs as well as nutrition, were identical; all the animals were normal. The

methods of experimentation were also identical and the only difference consisted in the different character of the cancer cells. The sarcoma cells injected into the circulation did not develop into a metastatic growth, while the adenocarcinoma cells did. The formation of metastasis upon the inoculation of the sarcoma into the liver and spleen was apparently due to the change of character which the sarcoma cells underwent during their sojourn in the parenchymatous organs. Such changes in the character of the cells after a lodgment in a different organ may occur also in human pathology, as was shown by Hansemann (9) and others.

In the present investigation the writer undertook to study the influence of the organism of the host upon the development of secondary metastatic tumors, while the character of the cancer cells used for the experiments was not changed. The research was conducted with the same two tumors of the white rat described above.

SUBCUTANEOUS INOCULATION AND SUBSEQUENT INOCULATION INTO A PARENCHYMATOUS ORGAN.

In a previous investigation (10) it was shown, in agreement with other investigators, that it is possible to inoculate successfully under the skin a tumor in an animal bearing in a different region a previously inoculated subcutaneous tumor. In order to approach more nearly the conditions obtained during the formation of metastasis, inoculations were made into a parenchymatous organ of animals in which a subcutaneous inoculation was done previously. In a former investigation the writer (11) has shown that when an animal is resistant to a subcutaneous inoculation of a tumor it will also resist in every case a subsequent inoculation into an organ. In the present study an inoculation was done into parenchymatous organs of animals of a tumor which was previously inoculated into the same animal subcutaneously.

With regard to the method used in the experiments the following explanation should be made. A normal rat, as has been shown by the writer in a number of investigations, stands a laparotomy and inoculation of a tumor into an organ very well and there is practically no postoperative mortality. But tumor animals are a

great deal more vulnerable, and a number of them succumb to the operation. A description of the experiments follows.

Experiment 1.[1] *Sarcoma in the Liver.*—A laparotomy was done on eighteen rats which were previously inoculated subcutaneously with a rat sarcoma. A small piece of the same tumor was introduced by the aid of a trocar needle into the liver. Of the thirteen animals that survived the operation for two weeks, six developed a large subcutaneous tumor and also a fairly large sized tumor in the liver. Figure 1 shows one of these animals. Seven animals developed only a small nodule subcutaneously and none of them developed any growth in the liver.

Experiment 2. Carcinoma in the Liver.—An inoculation of the adenocarcinoma into the liver was done on twenty-six rats which were previously inoculated subcutaneously with the same tumor. Sixteen animals survived the operation and of these five developed a large subcutaneous tumor and also a fairly large tumor in the liver. Eleven animals developed a small subcutaneous nodule and showed no growths in the liver.

Experiment 3. Sarcoma in the Spleen.—A laparotomy was done on nine rats which were previously inoculated subcutaneously with a rat sarcoma. A small piece of the same tumor was introduced into the spleen by the aid of a trocar needle. Of the six animals that survived the operation for two weeks, three developed a large subcutaneous tumor and a fairly large tumor in the spleen Three animals developed a small nodule subcutaneously and showed no growth in the spleen.

Experiment 4. Carcinoma in the Spleen. The same operative method was employed as in experiment 3. The carcinoma was inoculated in the spleen of six rats in which a subcutaneous inoculation of the same tumor was previously done. The three rats that survived the operation for two weeks developed a large subcutaneous tumor and a fairly large tumor in the spleen.

The results of the series of experiments with subcutaneous inoculations and subsequent inoculations into a parenchymatous organ are significant inasmuch as they clearly indicate the influence of the organism of the host on the growth of metastases. The subsequent inoculation into an organ of a tumor-bearing animal represents an artificially induced formation of a metastasis. The results of the present as well as the former investigations of the writer show that an inoculation into an organ will not succeed in an animal into which a previously attempted subcutaneous inoculation failed entirely or in which there developed only a small subcutaneous nodule. On the other hand, in the animals in which the subcutaneous inoculation produced the formation of a large subcutane-

[1] All the experiments were done under ether anesthesia.

ous tumor, the subsequent inoculation into an organ was also invariably successful. It is a well established fact that the success or failure of growth of a primary tumor inoculated in a normal animal is due to the conditions of general susceptibility or resistance of the organism of the animal to the growth of the tumor. Apparently the same general phenomena influence the growth of the secondary inoculated tumor, or, in other words, influence the formation of the artificial metastases.

SUBCUTANEOUS INOCULATION AND SUBSEQUENT SIMULTANEOUS INOCULATION INTO TWO PARENCHYMATOUS ORGANS.

The formation of metastases is a complex phenomenon. Not only does the frequency and localization of metastatic growth differ in the various tumors, but even the same tumor may form metastases in different organs in different individuals. In order to imitate experimentally this phase in metastasis formation, a simultaneous inoculation was done into two parenchymatous organs.

Experiment 5. Sarcoma in the Liver and Spleen.—The same operative methods were employed as in the previous experiments. A laparotomy was performed on sixteen rats which received two weeks previously a subcutaneous inoculation of the sarcoma. A small piece of the tumor was introduced subcutaneously into the liver and the spleen. Of the fifteen animals that survived the operation for two weeks, six developed only a small subcutaneous nodule and in these there was found no growth either in the liver or in the spleen. Nine animals developed large subcutaneous tumors, and of these, three showed a growth only in the liver, and three showed growth only in the spleen.

The results of this series of experiments show that, on the whole, the results of the multiple inoculations also depend upon the general conditions of resistance or susceptibility of the organism of the host. When the tumor fails to grow it fails everywhere, and when it succeeds, it succeeds in every organ. On the other hand, in those instances where the tumor grew both subcutaneously and in one organ and failed to grow in another organ there must have been an additional factor; namely, while the organism of the animal as a whole was susceptible to the inoculation both subcutaneously and into one organ, the other organ, the spleen or the liver, resisted the growth of the tumor. The possibility of such a local organ resistance in an animal of which the general organism is

susceptible to the growth of the same tumor was proven by the writer (12) in a recent investigation.

The Flexner-Jobling carcinoma of the white rat grows readily when inoculated anywhere in the organism, but fails to grow when inoculated in a normal testicle. But when an inflammatory lesion is produced in the testicle previous to the inoculation of the tumor, then the tumor inoculation is successful. The mechanism apparently causes the failure of growth of a tumor in one organ, while the same tumor grows subcutaneously and also in another organ.

SURGICAL REMOVAL OF A SUBCUTANEOUS TUMOR AND SUBSEQUENT INOCULATION INTO PARENCHYMATOUS ORGANS.

Uhlenhuth, Händel, and Steffenhagen (13) have shown recently that when a subcutaneous tumor is removed surgically and the same tumor inoculated subcutaneously in a different place, the second inoculation will not succeed if the removal of the first tumor was radical; but when there is a recurrence after the removal of the first tumor, then the second inoculation also succeeds. These striking results were confirmed by Meidner (14), Händel and Schönburg (15), and Uhlenhuth, Dold, and Bindseil (16). Apolant (17) seems to have reached different results and claims that there is no relation between the recurrence of the first tumor and the success of the second inoculation.

The influence of the surgical removal of a primary tumor upon the growth of metastases is of great practical and theoretical importance. In view of this and also of the contradictory results of the work of the different investigators it seemed advisable to repeat and enlarge upon these experiments by removing surgically the subcutaneous tumors and subsequently inoculating the same tumor into one or two parenchymatous organs.

Experiment 6. Carcinoma Removal and Inoculation in the Liver.—In ten animals a longitudinal incision was made at the inner side of the subcutaneous tumor and the latter was enucleated *en masse*. Immediately following this step the peritoneum was opened and a small piece of the animal's own tumor was inoculated into the liver. Of the five animals that survived the operation the primary tumor recurred in three, and in these there was a positive growth in the liver; in two animals there was no subcutaneous recurrence and no growth in the liver.

Experiment 7. Sarcoma Removal and Inoculation into the Liver and Spleen.—The method of operation was identical to the one described in experiment 6. In eleven animals the subcutaneous tumor was radically removed and then the same tumor was inoculated simultaneously into the liver and spleen. Of the ten animals that survived the operation, in seven there was no recurrence of the subcutaneous tumor and in these the inoculation into the organs failed invariably. In three animals the subcutaneous tumor recurred and of these animals in one the growth was positive in both liver and spleen (figure 2), in one growth was found only in the liver, and in the last animal a growth was found in the spleen.

Experiment 8. Carcinoma Removal and Inoculation into the Liver and Spleen.—The method of operation was the same as in the previous series of experiments. In twenty-three animals the subcutaneous tumor was radically removed and then the same tumor was inoculated simultaneously into the liver and spleen. Seventeen animals survived the operation and in eight of these there was neither a recurrence of the subcutaneous tumor nor any growth in the organs. In three animals there took place a recurrence of the subcutaneous tumor and positive growth both in the liver and the spleen. In the last six animals there was a recurrence of the subcutaneous tumor and a positive growth only in the spleen.

This series of experiments in the first place corroborates the findings of Uhlenhuth and his collaborators, that a subsequent inoculation succeeds whenever there is a recurrence of the primary tumor, and fails when the removal of the primary tumor remains radical. The experiments show further, like the previous experiments of the present study, the influence of the general and local resistance or susceptibility of the organism of the host on the growth of the secondary metastatic tumors. They also emphasize the relation between the postoperative recurrence of the primary tumor and the formation of metastasis. When the organism possesses a certain amount of resistance it will neutralize the few cancer cells which must necessarily be left behind after the apparently most radical operation; there will not take place any local recurrence and for the same reason the secondary or metastatic tumor will fail to grow. While, on the other hand, when this resistance of the organism is lacking, notwithstanding the most painstaking operation both a local recurrence and the formation of metastasis will take place.

The greater frequency of the growth of carcinoma in the spleen than in the liver may be due to the greater vulnerability of the former organ. The spleen of animals inoculated with the car-

cinoma frequently shows the condition of an amyloid degeneration, and, as has been shown by the writer in experiments with the testicle, a diseased organ is more susceptible to growth of tumor than a normal one. The writer is at present engaged in the study of this question.

The analysis of the results of the present investigation shows clearly that the differences in the frequency of the occurrence and in the specific localization of metastases are not due to the ease of detachment of the cancer cells from the primary tumor. Nor does the difference in the mode of transportation play a part. The main factors in determining the localization and frequency of metastases are the character and malignancy of the cancer cell on the one hand, and the general and local susceptibility of the organism of the host on the other. The failure or success of the proliferation of a group of cancer cells transported from the primary tumor into a distant organ is a result of the interaction of these two causes.

The validity of this conclusion will be further enhanced when it is considered that the mechanism and cause of growth of the primary tumor and the metastasis are identical. In inoculable tumors this identity is certainly complete since even the primary tumor is caused by the proliferation of a group of cancer cells introduced from outside. But even in a spontaneous tumor of the same animal, or in human cancer, the difference between a primary tumor and a metastasis consists only in the fact that in the former one must consider the additional unknown factor, namely, the transformation of a normal cell into a cancerous one. Otherwise the further growth and development of the primary tumor, as well as the metastasis, depends upon the correlation of the two factors mentioned above.

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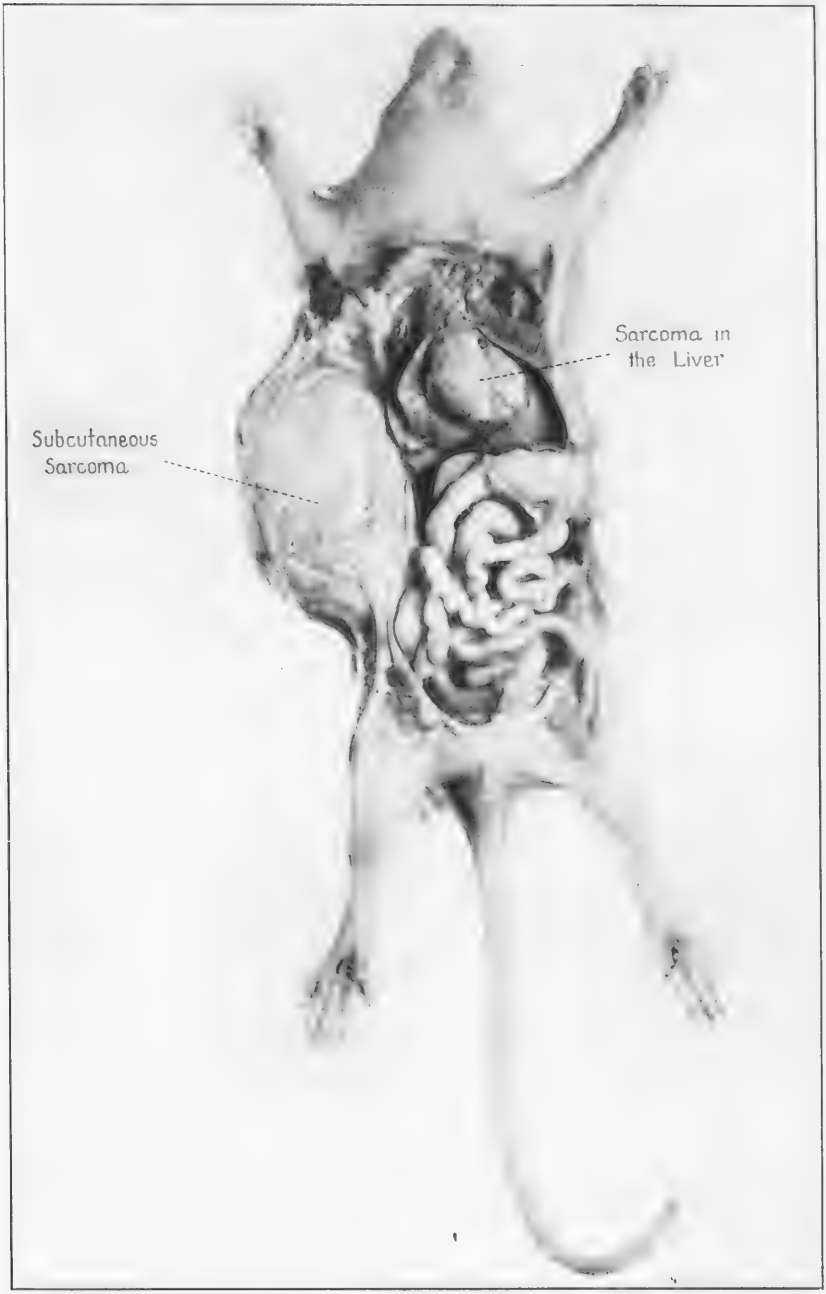

FIG. 1.

(Levin: Metastasis Formation in Experimental Cancer.)

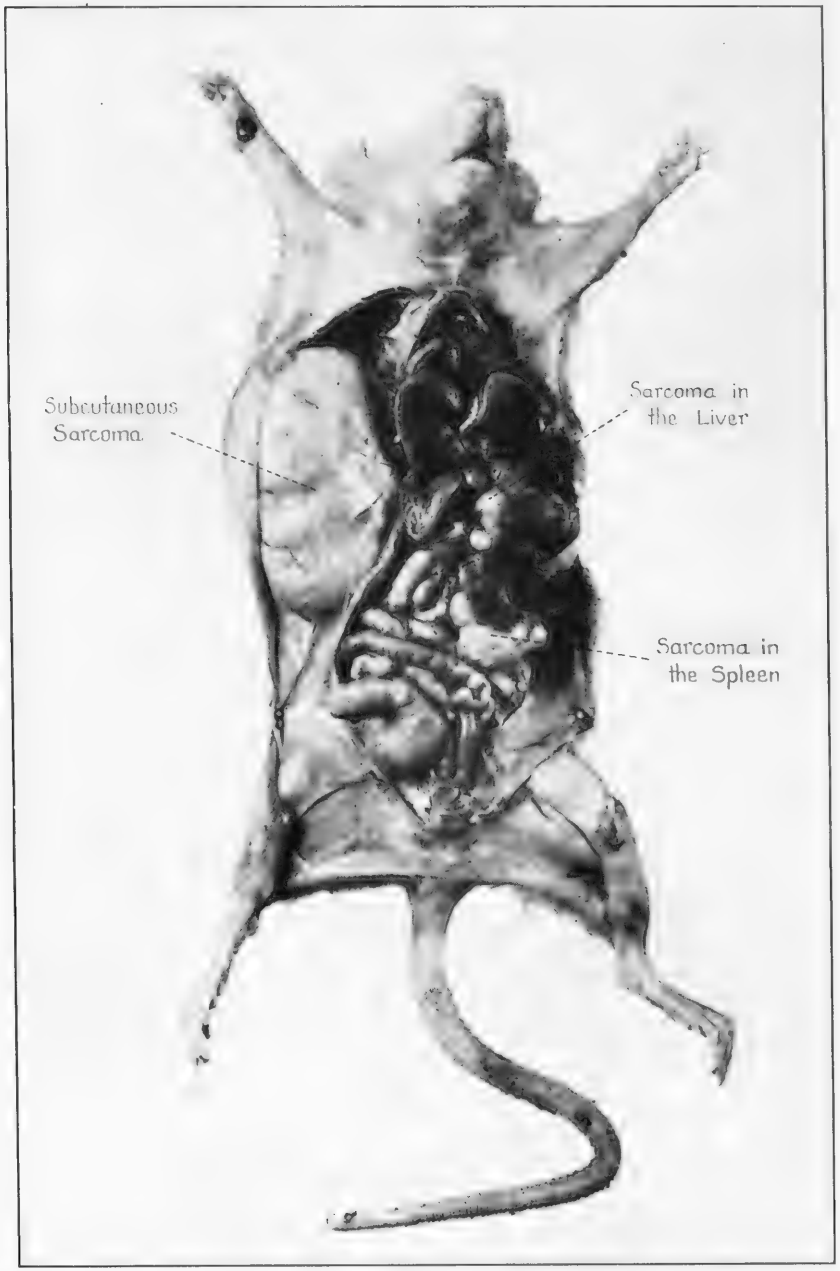

FIG. 2.

(Levin: Metastasis Formation in Experimental Cancer.)

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EXPLANATION OF PLATES.

Plate 25.

Fig. 1. Sarcoma of the white rat. A large subcutaneous tumor and a fairly large tumor in the liver.

Plate 26.

Fig. 2. Sarcoma of the white rat. A large subcutaneous tumor and a tumor growth in the liver and in the spleen.

THE INFLUENCE OF TEMPERATURE AND FLUID MEDIUM ON THE SURVIVAL OF EMBRYONIC TISSUES IN VITRO.*

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The object of this report is to present briefly the results of studies upon the duration of life of embryonic tissues preserved *in vitro* at varying temperatures and in several fluid media, the method of tissue cultivation being used to determine the viability of the tissue.[1]

There are many reports in the literature concerning the survival of tissues and organs after removal from the body, especially of the tissues which are frequently transplanted surgically in human beings. Wentscher (1), for example, was able to graft pieces of human skin preserved for twenty-two days *in vitro;* Ljunggren (2) obtained positive results with skin kept for thirty days in tubes of ascitic fluid; while Carrel (3) reported recently that he had been able to transplant to other individuals pieces of skin removed from a child, after two to seven weeks' preservation in cold storage; the pieces preserved for the longer period, however, were not uniformly successful.

Similar reports have been made regarding the preservation of other tissues. Tuffier (4) transplanted omentum kept for one month in cold storage; Magitot (5) grafted corneas which he had preserved for eight days; and Davis (6) obtained good results with fascia transplanted after being stored for forty days at 32° C.

In all these instances the survival of the organs or tissues was indicated by the success of the transplantation. A clinically successful transplantation does not, however, prove that the transplanted cells were living, for Levin and Larkin (7) and Carrel (8)

* Received for publication, June 5, 1913.

[1] For details of the technique of the method, see Lambert, R. A., and Hanes, F. M., *Jour. Exper. Med.*, 1911, xiii, 495; xiv, 129.

have shown that segments of killed blood vessel may be successfully grafted, the dead tissue affording a temporary but competent tube which is gradually replaced by the tissues of the host. It may nevertheless be assumed that many adult tissues, especially skin and structures of a purely connective tissue type, are able to survive, under suitable conditions, for days or even weeks after removal from the body. But this may not be true of embryonic tissues. Indeed, Askanazy (9) found in his transplantation experiments that the tissues of rat embryos kept in the ice box for longer than eight days gave regularly negative results.

In our investigations, in which the tissues of chick and rat embryos have been used, the results as regards the possible duration of life *in vitro* and the influence of certain factors (temperature and fluid medium) on this period of survival have been so definite that it seems probable that the points brought out may have a more general application. It is rather on this account than because of the interest of the results themselves that this report is made.

EFFECT OF TEMPERATURE.

The technique used in these studies was quite simple. Small pieces of the tissue (chick or rat embryo) were preserved generally in hanging drop plasma preparations, which were carefully sealed with vaselin. The slides were divided into groups and stored at different temperatures. After varying periods of time the slides were removed, the pieces of tissue were transferred to fresh drops of plasma, and the new preparations were incubated at 37° C. This method of preserving the tissue in hanging drop slides is especially convenient and reduces to a minimum the opportunity for infecting the tissue in handling.

Two refrigerator ice boxes were used in the majority of the experiments. The temperature in them varied somewhat in different seasons, but for periods of one to three weeks the variations were not, as a rule, greater than 2° to 3° C.

The first series of experiments was carried out in midwinter when the temperature of one of the ice boxes was as low as —6° to —7° C. A series of slides containing pieces of chick embryo was

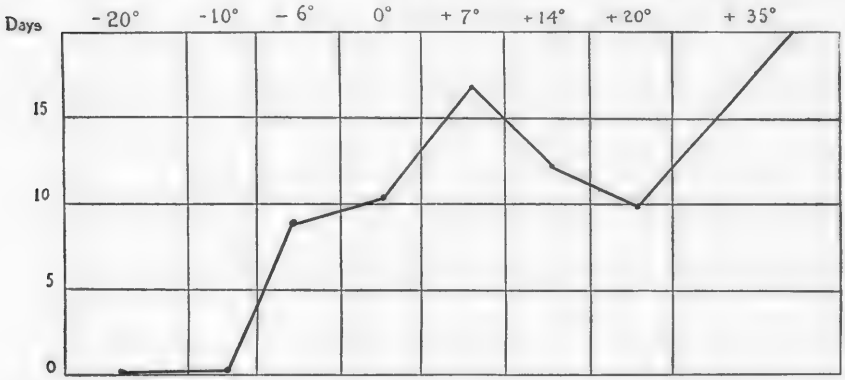
stored at this temperature; another series was placed in a north room without a radiator, in which the temperature during the course of the experiment ranged between + 12° and + 14° C. After periods of from two to sixteen days, pieces of tissue from both series were transferred to fresh drops of plasma and incubated at 37.5° C. Since the freezing point of chick tissue is about — 7° C., some of the ice box specimens were found to be frozen. The tissue from none of these grew upon incubation. Tissue from the unfrozen preparations preserved as long as six days at this temperature exhibited active growth upon incubation. Eight day preparations gave only occasional growths, while tissue preserved ten days or longer at this temperature gave uniformly negative results. Tissue kept eight and ten days at room temperature, however, showed active growth, as did also, though less regularly, twelve day specimens; sixteen day preparations were uniformly negative. Repetitions of this experiment gave practically the same results.

It was concluded, therefore, that chick tissues may be preserved longer at cool room temperature than at a temperature just above the freezing point of the tissues, and, further that the limit of survival even at the more favorable temperature is relatively short (twelve to fourteen days).

A second series of experiments was carried out later when the temperature in the two refrigerator ice boxes was respectively — 1° to 0° C. and + 6° to + 7° C. The temperature at this time in the room used varied from + 18° to + 21° C. The tissue was preserved, as before, in the form of fairly small fragments in plasma sealed in hanging drop slides. Transfers were made after varying intervals up to twenty days as in the previous experiment. The results may be briefly stated as follows: The tissue preserved for eight days at — 0° to — 1° C. showed active growth upon incubation; ten day preparations were viable although many of the transfers were negative, and the positive cultures exhibited a relatively long latent period (two to three days). Tissue preserved for a longer time at this temperature failed to grow. Tissue preserved at + 6° to + 7° C., on the other hand, was regularly found to be viable after twelve to fifteen days, and some of the sixteen and eighteen day preparations gave a fair growth upon incubation.

Tissue preserved at + 18° to + 21° C. survived for from nine to eleven days; the tissue kept for a longer time failed to grow.

Stated summarily, then, it was found that of specimens of chick tissue kept at temperatures around —6°, 0°, + 6°, + 14°, and + 20°, those at + 6° survived longest; temperatures around 0° and + 14° were about equally favorable. Since chick tissues survive freezing at —8° to —10° for a few hours at most, and exposure



TEXT-FIG. 1. Chart showing the approximate periods of survival of embryonic tissues preserved at different temperatures between —20° and + 35° C. Above 25° C. chick tissues exhibit *in vitro* active growth which may be maintained for an indefinite time. The last segment should therefore be regarded as incomplete.

to —20° for a few minutes only, and since at incubator temperatures (30° to 37°) the same tissue may be preserved in a state of active life for many weeks or even months, a curve plotted to show these variations in length of life at different temperatures would present first almost a horizontal line, then a sharp rise, then a gradual rise, then a slow decline, followed by a second rise (text-figure 1).

The experiments upon the effect of temperature were repeated with rat embryonic tissues instead of chick tissues. The findings were in general the same as with chick tissue. At 0° the period of survival was about ten days; at + 6°, a few days longer. Rat tissues were more readily killed by freezing.

EFFECT OF FLUID MEDIUM.

It occurred to us that in regard to the medium used there might be at least two factors influencing the duration of life of the tissue: first the kind of medium, and secondly the quantity of fluid used in proportion to the amount of tissue. Four isotonic fluids were used as media: plasma, unmodified or diluted blood serum, Ringer solution, and physiological salt solution (0.85 per cent. sodium chloride). The same technique was used as in the temperature experiments, except that in some instances instead of making hanging drop preparations, the tissue and fluid were placed in the bottom of the cavity slides and sealed as before. The specimens were kept at 0° and + 6° C.

It was found, in brief, that the duration of life was not appreciably influenced by the use of the different fluids. It seemed conceivable, however, that although the kind of isotonic medium might be without effect, the relative quantity of fluid used and the size of the tissue fragments might exert an important influence. It seemed logical to suppose that a larger quantity would allow the dilution of the waste products of tissue metabolism, thus favoring a longer period of survival. To test this hypothesis small pieces of embryo were preserved in dishes containing 50 to 100 cubic centimeters of Ringer solution or diluted blood serum, and entire embryos were placed in another dish containing only enough fluid to cover the surface of the embryo. Pieces of tissue were also placed in dishes wrapped in gauze moistened with Ringer solution.

Subsequent transfers of the tissue showed little or no difference in the duration of life under the several conditions. In one experiment the tissue preserved in the form of entire embryos with very little fluid survived three days longer than the tissue divided into small fragments in a large dish of fluid, a result contrary to what our working hypothesis led us to expect. It seemed probable that the better preservation in the first case was not due to the smaller amount of fluid used but to the slighter mechanical injury which the larger piece of tissue suffered in handling.

CONCLUSIONS.

1. Embryonic chick and rat tissues preserved at temperatures ranging from $-7°$ to $+20°$ C. live longest at about $+6°$ C. The duration of life under the most favorable conditions is less than twenty days.

2. The kind of isotonic medium used,—plasma, serum, Ringer solution, or normal salt solution,—does not appreciably influence the period of survival. The quantity of medium in proportion to tissue is similarly without marked effect.

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REGENERATION OF AXIS CYLINDERS IN VITRO.

Second Communication.*

By RAGNVALD INGEBRIGTSEN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

Plates 27 to 31.

In a previous article[1] I described certain observations on cultures made from pieces of the central nervous system of young mammals, cultivated in coagulated plasma. Most of the observations were made from living (unstained) material. Further study of the cultures, carried on with different methods of fixation and staining, has confirmed the observations made on unstained preparations; namely, that nerve fibers grow out from pieces of the cerebellum and spinal ganglia of young mammals, when cultivated in plasma. Certain other phenomena of growth have also been revealed. This paper, however, will be limited to a description of the outgrowth of nerve fibers and their appearance in stained specimens.

As young dogs were not available, young cats and guinea pigs were employed in most of the experiments. The cultures were chiefly made from pieces of cortex of the cerebellum and spinal ganglia.

Held's pyridin method was found to be the only practicable one for fixing the cultures. It causes no precipitation in the plasma, and the subsequent staining by Cajal's silver nitrate method, when successful, has given good results. In good preparations the fragment of tissue stains dark brown or black and the surrounding plasma a light reddish brown. The black or dark brown nerve fibers which have grown out are easily discernible against this background.

Cerebellum.—The network of dark brown or black nerve fibers

* Received for publication, June 17, 1913.

[1] Ingebrigtsen, R., *Jour. Exper. Med.*, 1913, xvii, 182.

contained in the tissue can be observed in the thinner part of the transplanted fragment, especially along the border lines adjoining the plasma. In some preparations the new fibers in the plasma can be determined as a direct outgrowth or continuation of fibers contained in the tissue itself (figure 1). In other preparations this direct connection is not visible, either because the original fragment fails to reveal individual fibers or because it is surrounded by other newly grown tissues (connective tissue, glia), which cover the nerve fiber at the point where it emerges from the brain and enters the plasma (figure 2).

From this point the nerve fiber often proceeds by means of a terminal bulb, which in the living culture possesses ameboid movements. In stained preparations the terminal bulb usually takes on a somewhat lighter shade of brown than the fiber itself and shows an even, slightly granular structure. It is ovoid or globular in shape with a diameter of two to three microns. No capsule has ever been seen surrounding it. This end bulb is not, however, invariably present; sometimes the fiber proceeds with a point, and in one preparation the swelling at the end of the fiber is probably produced by a minute loop formed by its point (figure 3).

Throughout their course the fibers are either evenly cylindrical (figure 2) or provided with varicosities, which have much the same appearance as the terminal bulb (figures 1 and 4).

Spinal Ganglia.—While the cultures of cerebellum in this series of experiments with stained specimens have shown only a scant number containing newly grown nerve fibers, the cultures of spinal ganglia give better results both as regards the number of successful cultures and the number of nerve fibers growing out in each culture.

The technique employed for the preparation was the same in both cases: the pieces of cerebellum, as well as of the spinal ganglia, were taken from the living etherized animal, put directly into Ringer solution, where they were cut, and from there transferred to the plasma.

The success of the operation depends upon the rapidity with which it is carried on, at least in the case of the cultures of the cerebellum, twelve to fifteen seconds being the longest lapse of time permissible between the removal of the tissue from the animal and

its inoculation into the plasma. In the case of the spinal ganglia the interruption of the circulation and the transplantation to the new medium is more easily withstood without injury.

Most of the cultures of spinal ganglia, as well as those of cerebellum, were fixed and stained after two or three days' incubation, in order to be sure that degenerative changes of the newly grown fibers had not occurred; as a rule, these changes begin to appear on the fifth and following days at an incubation of 39° to 40° C. On the other hand, the growth of the nerve fibers is most rapid during the first two days, and on the second day, in particular, they attain, if not their full size, at least almost their entire length.

In the cultures of spinal ganglia, as well as in those of cerebellum, the nerve fibers in the plasma in several preparations have grown out as a direct continuation of the fibers in the original piece, and the outgrowth of new fibers is especially abundant in places where parallel and transversely cut nerve fibers radiate out against the plasma. The nerve fibers are also seen to grow out from masses of ganglia cells, situated at the edges of the fragment (figure 5).

The nerve fibers growing out from the spinal ganglia generally reach a greater length than those from the cerebellum, many of them attaining 500 to 600 microns after forty-eight hours. The different fibers vary somewhat in thickness, and the heaviest ones branch several times. They are mostly cylindrical and absolutely even throughout their course, but they bend and curve in various directions, and sometimes spirally. Most of them end in a point. In one preparation most of them end in a bulb, and in this case the fibers are at intervals provided with varicosities, spindle-shaped, ovoid, or globular in form. These have a slightly granular structure and the centers stain somewhat darker than the peripheral parts.

In none of the stained cultures, whether taken from the cerebellum or the spinal ganglia, have I observed a true anastomosis. In the previous paper I described the existence of anastomoses between some of the fibers in the living cultures. This phenomenon has not been confirmed in the stained specimen, and its interpretation will therefore require further study. It may be merely a coalescence between two fibers situated closely together.

The nerve fibers from the cerebellum, as well as those from the

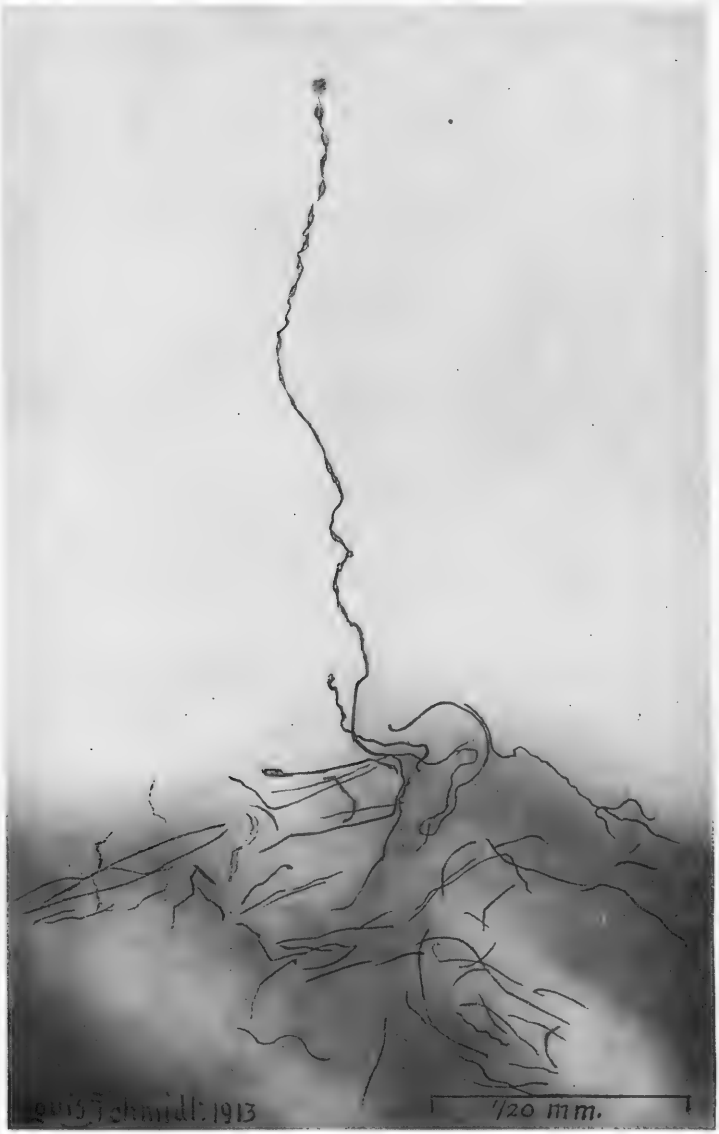

FIG. 1.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

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FIG. 2.

Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

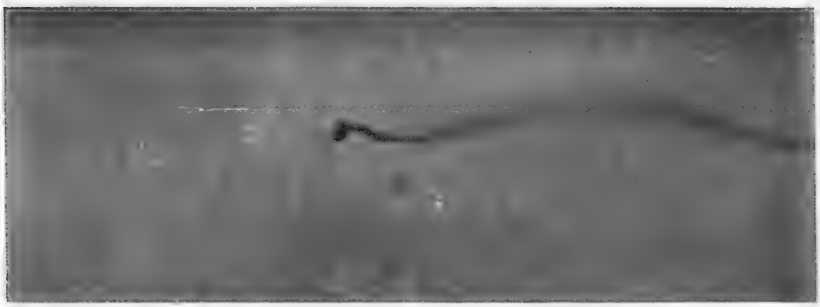

FIG. 3.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

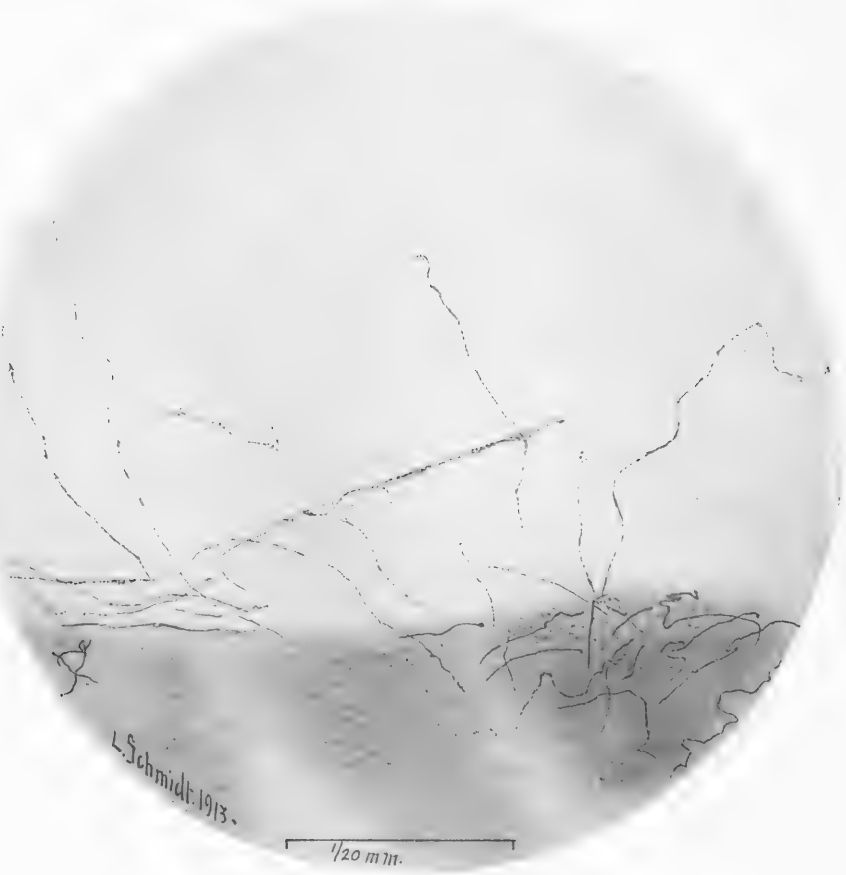

FIG. 4.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

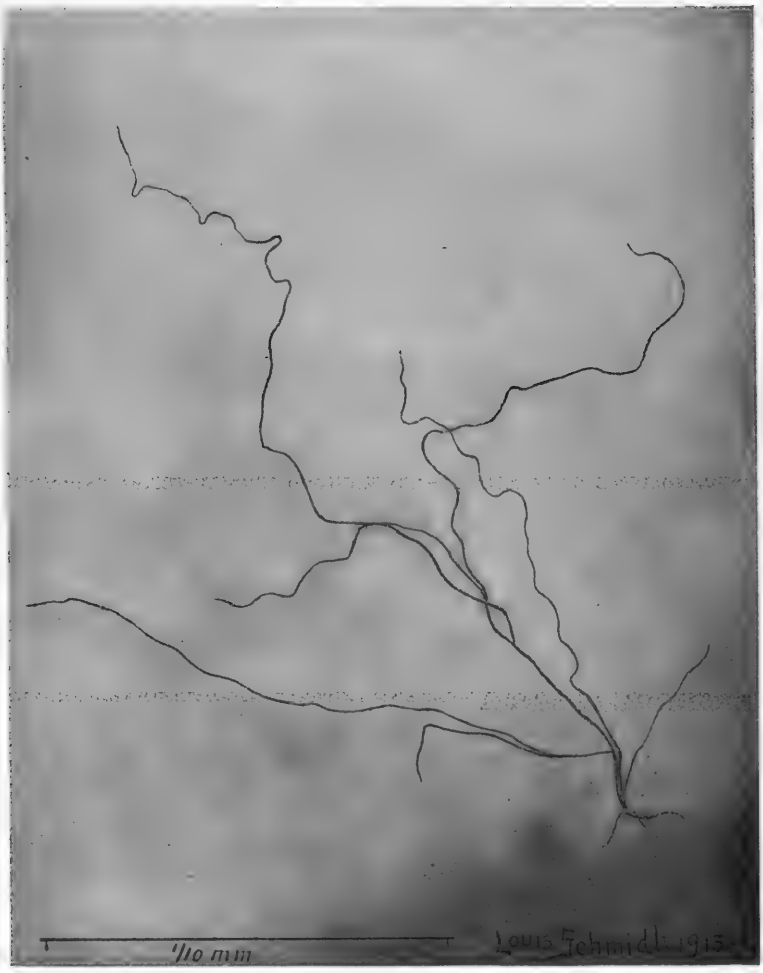

FIG. 5.

Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

spinal ganglia, emanate freely into the plasma without the support or attachment of any other structure. They are not accompanied by other tissue; sometimes they are surrounded by connective tissue cells or glia cells, but their growth begins earlier and does not depend upon the presence of these structures. In fact, cultures with rich growths of connective and glia tissues rarely contain newly grown nerve fibers.

SUMMARY.

It has been shown for the first time that nerve fibers grow out from pieces of cerebellum of young cats and guinea pigs, when cultivated in coagulated plasma.

The same phenomenon has been observed in cultures of spinal ganglia.

The nerve fibers do not anastomose and they extend into the plasma unaccompanied by structures of any kind.

EXPLANATION OF PLATES.

Figures 1, 2, 4, and 5 are camera lucida drawings focused on different planes of the plasmatic medium, in order to bring out the nerve fibers throughout their entire length.

PLATE 27.

FIG. 1. Two day old culture of cerebellum of a guinea pig eight days old.

PLATE 28.

FIG. 2. Two day old culture of cerebellum of a guinea pig eight days old. A single nerve fiber is seen surrounded by other structures, probably neuroglia.

PLATE 29.

FIG. 3. Photographs of a two day old culture of cerebellum of a cat six days old. The photographs are taken at two different magnifications, 1,300 and 1,900 diameters respectively.

PLATE 30.

FIG. 4. Two day old culture of cerebellum of a guinea pig eight days old.

PLATE 31.

FIG. 5. Two day old culture of spinal ganglia of a guinea pig six days old.

RESISTANCE TO A TUMOR-PRODUCING AGENT AS DISTINCT FROM RESISTANCE TO THE IMPLANTED TUMOR CELLS.

Observations with a Sarcoma of the Fowl.*

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(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

Plate 32.

No one of the problems opened to experiment by the transplantation of animal tumors has received more attention than that of the nature of resistance to these growths. The exact manner of the resistance, or resistances,—for there may well be several kinds,—is still undetermined; nevertheless one important fact has emerged, namely, that the fate of implanted tumor, as indicating this resistance, is influenced for the most part by the same conditions that influence the fate of implanted normal tissue. The conditions at the site of the graft's implantation, the age of the host, its health, species, even variety, and its relationship by blood to the previous host,—these and other factors influence in like manner engrafted tissue of both sorts.[1] Furthermore, resistance to transplanted tumor can be induced by a preceding injection of living tissue,[2] and whether this tissue be normal or neoplastic makes little difference.[3] By the same means too animals can be made resistant against implanted normal tissue which ordinarily would grow in them, for example embryonic tissue, so that it now fails to develop (Fichera, Rous). The numerous data thus far obtained go to show that the

* Received for publication, June 21, 1913.

[1] Loeb, L., *Proc. Am. Phil. Soc.*, 1908, xlvii, 3. Fichera, G., *Arch. ed atti d. Soc. ital. di Chir.*, 1909, xxii, 1. Rous, P., *Jour. Exper. Med.*, 1910, xii, 344.

[2] Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therap.*, 1906, No. 1, 77.

[3] Bashford, E. F., Cramer, W., and Murray, J. A., *Brit. Med. Jour.*, 1906, i, 207. Schöne, G., *München. med. Wchnschr.*, 1906, liii, 2517.

resistance which in some individuals prevents the growth of implanted tumor is a resistance directed against the graft as strange tissue, and is unconnected with the neoplastic qualities which this tissue happens to possess.

These are the findings with transplanted neoplasms. Of the specific factors concerned in resistance to the growth of spontaneous tumors, as distinct from non-specific factors, such as infection, mechanical disturbance, etc., practically nothing is known. Manifestly these growths cannot be looked upon as consisting of a tissue strange to the body in the sense that transplanted tumor tissue is strange. The not infrequent occurrence of spontaneous tumors in animals highly resistant to implanted growths (Bashford) proves this.

The present paper is a report of an attempt to learn, through the study of an avian tumor, something of the nature of resistance to spontaneous growths. The tumor employed is a spindle-celled sarcoma of the chicken, which has already been reported upon several times from this laboratory.[4] The growth, when transmitted by transplantation, behaves exactly like the transplantable tumors of mammals, being influenced by the factors which influence them in common with transplanted normal tissue. But from it there can be separated by drying, or filtration, or glycerinization, an agent, presumably living, which, under special conditions, will cause a sarcomatous change in the tissue of a previously normal fowl. The growth thus generated is derived from the host's own tissues and in this regard resembles the spontaneous tumors of mammals. A study of the forces influencing such a growth's development, and the circumstances which sometimes bring about its retrogression may well have a bearing on similar problems in mammalian oncology.

Natural retrogression of the spontaneous tumors of mammals is relatively rare; but retrogression can be induced, more or less successfully, by the Roentgen rays, radium, and ultraviolet light. The point of attack of these forms of radiant energy is still unknown. In the case of the chicken sarcoma when influenced by radiant

[4] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; for references to other articles on the growth, see Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, 1913, l, 637.

energy there arises at once the question whether the sarcomatous cells as such are primarily affected, or whether the agent responsible for the growth is injured, with a resultant lessened malignancy of the cells so that they can be destroyed by the body. My first experiments have been directed to this matter. Certain differences in the time of origin of growths caused by the dried tumor tissue, that is to say by an action of the tumor-producing agent, and of those resulting from the fresh tissue containing transplantable tumor cells have suggested the method of work.

METHOD.

With parallel safety-razor blades the fresh sarcomatous tissue is cut into a number of slices of equal thickness, ordinarily 0.08 to 0.1 cm. These are placed in Ringer's solution, carefully trimmed free of all except sound tumor, and are then separated into a number of batches. If the tissue is to be submitted to the Roentgen ray the slices of each batch, spread flat, are together sealed in a large hollow-ground slide under a large, thin cover-glass. If the ultraviolet ray is to be used the slices are repeatedly washed with Ringer's solution to rid them as far as possible of free albuminous matter, and, covered with a thin layer of the fluid, are exposed to the light in an open dish; or without the fluid they are sealed between two flat pieces of quartz glass previous to exposure. The control is treated in the same way except that it is not irradiated. Radium has not been employed thus far.

When the exposures have been completed the tissue is cut fine with sterile knives. In some of the experiments a little sterile infusorial earth was added to it. By means of trocars small portions (about 0.01 c.c. each) are now inoculated intradermally in the feather-free pectoral strip of a number of chickens. The remainder of the tissue is spread very thin in a dish, and placed *in vacuo* over sulphuric acid for twenty-four hours, which is sufficient time to render it completely dry. It is then restored to approximately its former bulk by the addition of an excess of Ringer's solution, or a very little distilled water, and inoculations are made with it as on the preceding day, using the same chickens and the feather-free strip of the other breast. Usually five batches of irradiated tissue and a control batch have been used, fresh and dry, making in all twelve inoculations to each fowl. The tissue bits are implanted several centimeters apart, in a line, and their order is varied from fowl to fowl. The tumors resulting are extremely discrete and are plainly visible at all stages of their development.

An Heraeus mercury-quartz lamp of 220 volts supplied the ultraviolet rays. The specimens were exposed at 25 cm. distance. The temperature of the preparations was at no time above 28° C.

For the Roentgen rays one or another of three soft tubes with a spark gap of 1.5 to 4.5 cm. has been employed. The specimens were placed within 5 to 10 cm. of the tube.

Five sets of observations with the ultraviolet rays and four with the Roentgen rays have been made by this method. For each experiment three to seven fowls were used, making in all forty-one inoculated, of which forty developed tumors. Retrogression soon occurred in some.

The growths from the inoculation of fresh material in intradermal sites arise in general almost immediately, resulting, as previous work has shown,[5] from an extremely rapid proliferation of the implanted cells. At the end of a week the little tumors may be one centimeter in diameter. They appear as raised, translucent bosses or buttons over which the epidermis is tense and smooth (figures 1 and 2). At the end of two or three weeks they very commonly ulcerate, or coalesce, so that observations beyond this period are not of great value. The growths from the dried material are exactly similar but appear much later, not until at least seven days have elapsed, and often twice or thrice this time. That desiccation completely kills the tumor cells seems certain from the findings of previous workers with the normal and neoplastic cells of the higher animals. Moreover, experiments by Dr. Murphy show that the embryonic tissue of the fowl does not survive drying; and drying renders completely innocuous another transplantable chicken tumor (Chicken Tumor XVIII), propagated in this laboratory.

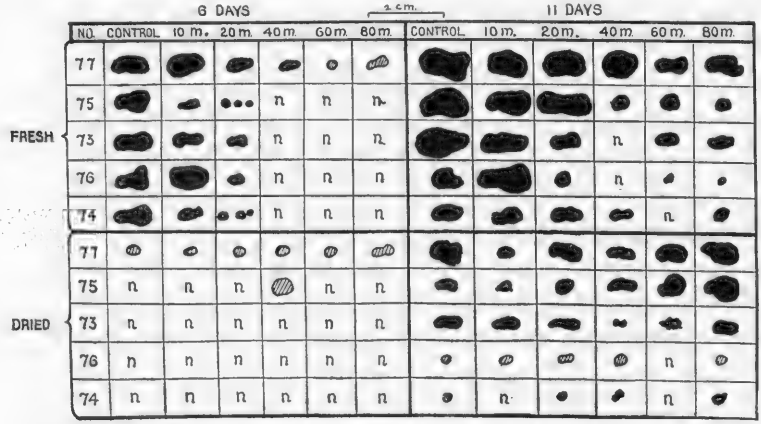
EFFECTS OF THE IRRADIATION.

The experiments have given almost diagrammatic results (text-figures 1 to 6). The Roentgen rays do not appreciably affect either the sarcoma cells or the growth's causative agent when the tissue has been exposed for eighty minutes, the longest time employed. This failure to be influenced is not surprising when it is considered how refractory many mammalian sarcomata are to the rays. The protocols of the individual experiments will not be given.

Ultraviolet light rapidly destroys the activity of the sarcoma cells and this without notably injuring the agent associated with them. In text-figure 1 its selective action is shown. At the end of six days good sized growths are found as the result of inoculation of the fresh control material, while the irradiated fresh tissue mani-

[5] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

fests less tumor-producing activity, until with sixty and eighty minutes' exposure this is completely suppressed. The dried material has as yet given rise to no growths. At the end of eleven days the effects of irradiation and of drying are less marked. In four of the five fowls the dried material has given rise to growths; and in the size of these growths there is no evidence of influence of the irradiation. Differences among the tumors from the fresh material still

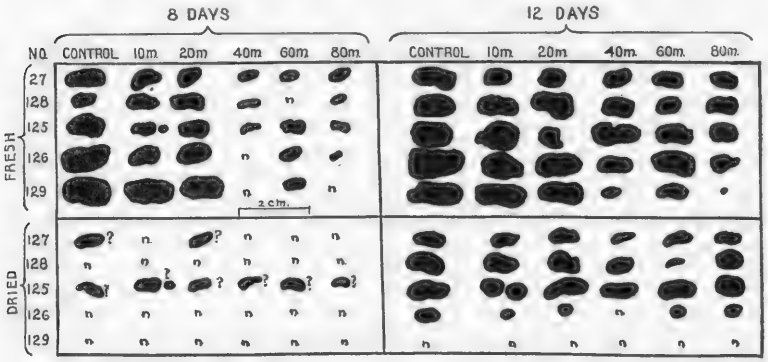


TEXT-FIG. 1. To show the effect of ultraviolet light on the activity of fresh and dried tumor tissue. In the first vertical column are the numbers designating the fowls. Then follow silhouettes to scale of the tumors. The measurements were taken six and eleven days after the inoculations. 10 m., 20 m., 40 m., etc. = 10, 20, and 40 minutes' exposure; n = no tumor. Cross-hatching = induration.

It will be seen that the irradiation has a marked injurious effect on the element in the fresh tissue (the living tumor cells) which gives rise to tumors immediately after implantation. The tumor-producing agent which resists drying is unaffected by the irradiation.

indicate this influence, but less strikingly than before, since now at length the material irradiated for sixty and eighty minutes has given rise to growths. These growths have arisen after about the same time and are of about the same size as those derived from the dried material. In text-figure 2 similar findings are presented but the differences are not so clear cut, since the longest period of irradiation has failed to rid the fresh tissue of the activity specifically associated with its fresh state.

The interpretation of these results is plain. The text-figures show, first, that in the sarcomatous tissue there are two elements capable of producing the growth, one of which will withstand drying while the other will not. The labile element, which we know to be the living and transplantable tumor cells, is so sensitive to the ultraviolet rays that sixty to eighty minutes' exposure will completely destroy its activity; whereas the stable element, the tumor-producing agent, is at most only slightly affected by this irradiation.



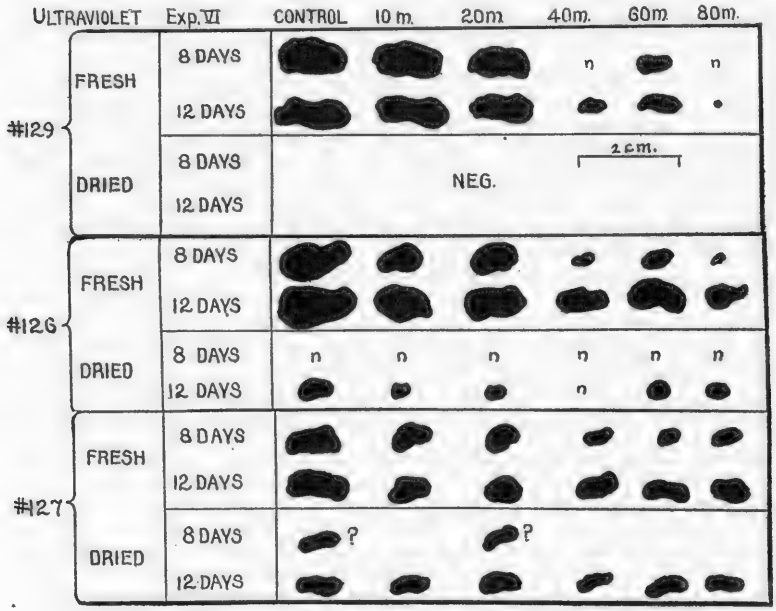
TEXT-FIG. 2. This chart is arranged in the same way and shows findings similar to those of text-figure 1.

TYPES OF RESISTANCE TO THE SARCOMA.

Taken from another point of view the irradiation experiments have demonstrated the presence in the fowl of two distinct resistances to the chicken sarcoma, directed, the one against the tumor cells, the other against the tumor-producing agent. These may exist separately or in one host.

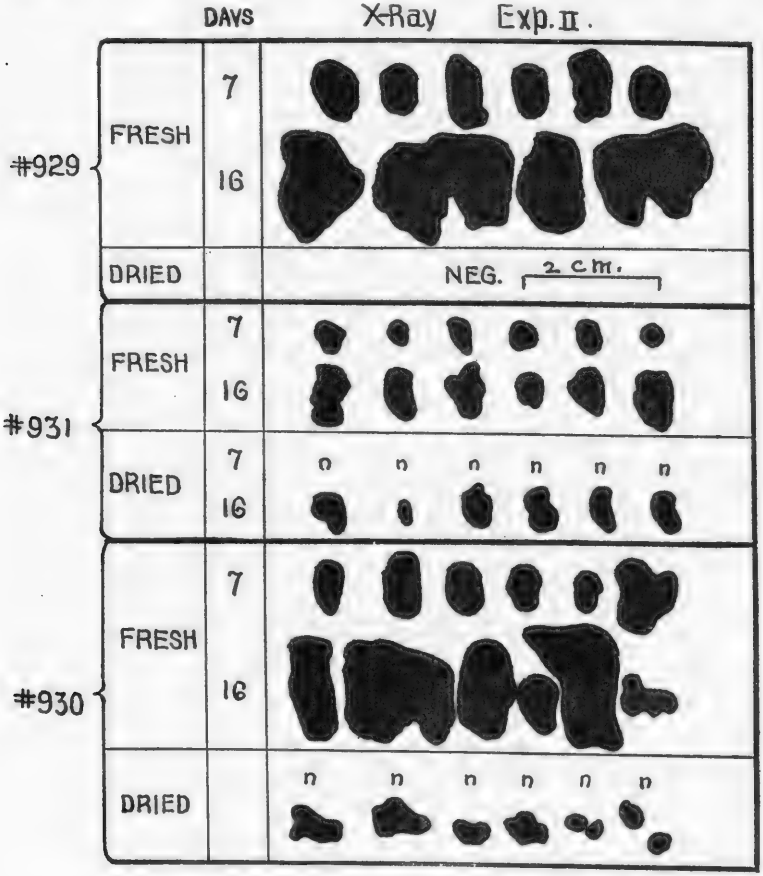
In text-figure 3 are given the findings in three of the fowls of text-figure 2 but now so arranged as to bring into contrast the fate of the fresh and dried material in the individual host. The fresh material produced tumors in all three fowls but with a very different degree of success. In one instance, No. 129, in which it gave rise to large tumors the dried material failed to engender growths, and in another, No. 126, this latter acted only slowly. In the remaining

instance growths developed rapidly from the dried material and the fresh material proved only slightly more active, the results with it being poor as compared with those in the first two hosts. In text-figure 4 precisely the same facts are shown of some fowls of X-ray experiment II, and here the complicating effect of a variation in tumor size due to injury from the irradiation is not



TEXT-FIG. 3. Some of the results shown in text-figure 2, but now so arranged as to demonstrate individual differences in the resistance of the fowls and the presence of two sorts of resistance. In No. 129 the fresh tissue rapidly gave rise to tumors, whereas the same material, when dried, failed to engender them. That this is not due to lack of activity on the part of the dried stuff is shown by the findings in the other two fowls. In fowl 126 the fresh tissue rapidly engendered tumors and the dried material in due time caused them. In No. 127 the dried material was active in the production of growths, whereas the fresh material was much less successful than in the fowls previously mentioned. It is evident that some hosts have a relative resistance against a tumor-producing element in the fresh tissue, while in some a resistance is directed against the tumor-producing element which survives drying. ? = possibly a tumor, possibly only induration.

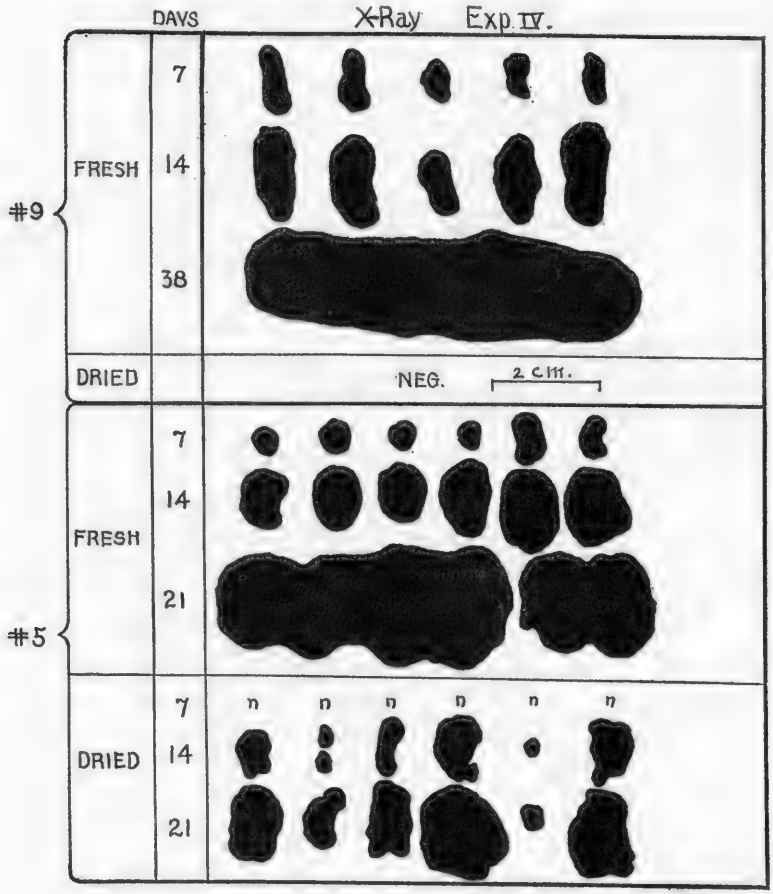
present. In text-figures 5 and 6 some phases of the matter are again illustrated. The fowls in which the dried material is very active



TEXT-FIG. 4. This illustrates the same facts as text-figure 3. The periods of exposure to the X-rays are not given since the material was practically unaffected by them.

are not necessarily those in which the fresh material does badly, but often ones in which it does very well. Other charts showing the same facts might be given. The chickens of all the experiments

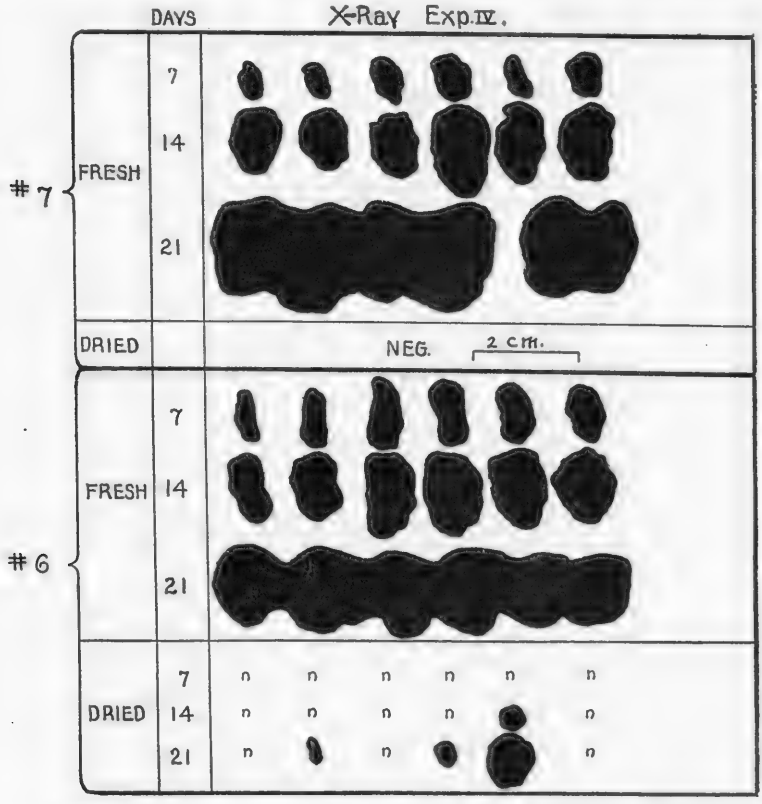
can be separated into four classes: those in which the fresh material gave rise to tumors and the dried gave none; those in which both



TEXT-FIG. 5. This illustrates further some of the facts of text-figures 3 and 4 and shows that good growth from dried material is not necessarily associated with poor results from the fresh tissue.

gave rise to tumors with nearly equal facility; others again in which neither gave rise to growths; and finally a majority in which both

inoculations resulted in growths, those from the fresh material appearing earlier and maintaining a marked general advantage of size. In other words the chickens showed themselves resistant or susceptible in widely various degrees to one, or another, or both the



TEXT-FIG. 6. This chart shows again the differences in individual fowls.

labile and stable elements going to produce the tumor. And we know that these labile and stable elements are, respectively, the tumor cell and the tumor-causing agent.

A number of questions at once present themselves in relation to this conclusion. Resistance to the transplanted tumor cells is never

so clear cut as that to the agent. It is at best only relative. If fowls are really resistant to the implanted cells how does it happen that even in hosts most resistant to them growths from the fresh material appear somewhat earlier and at first are larger than those from the dried material? One reason is because in resistant fowls a profuse, local, round-celled reaction occurs about the fresh tissue implanted in intradermal sites, forming transiently a considerable nodule.[6] Perhaps also the tumor-producing agent is somewhat attenuated by drying. Theoretically the findings, in fact all of the differences between fresh and dried material, might be explained on the assumption that the chicken sarcoma is never really transplanted but comes only from infection by means of an agent largely impaired by drying. This is contrary to the facts and entails awkward secondary assumptions, for example, that fowls react differently to the stable and labile portions of the agent, some being susceptible only to the one, some to the other. But we know that the differences between fresh and dried material are primarily those of the tumor cells involved. Furthermore, investigations have shown that some fowls are naturally resistant to the action of the tumor-producing agent[7] and some to growth of the implanted tumor cells.[8] The present work goes only a step further in demonstrating that the resistances thus manifested are independent of one another.

It is interesting to consider in the light of these results the rôle of the causative agent in the growth of the sarcoma in the individual fowl. Histologically there is no suggestion that the agent takes part in this process. And yet in one class of susceptible fowls, as the present results show, it doubtless aids in the growth's extension. In another class the tumor's development following the implantation of tumor cells is probably from the first solely the result of the proliferation of these cells.

In the present state of our knowledge it is impossible to say whether findings with chicken tumors have a direct application to

[6] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

[7] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

[8] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr., loc. cit.*

FIG. 1.

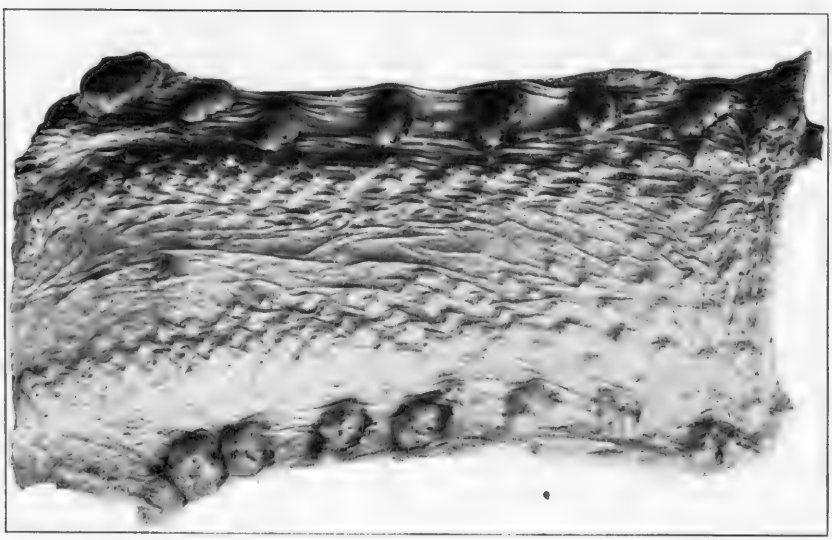


FIG. 2.

(Rous: Resistance to Tumor-Producing Agent.)

the problems of mammalian tumors. Were this so then the present observations would go far to explain how it happens that "spontaneous" growths can arise, as they sometimes do, in rats or mice highly resistant to transplanted neoplasms. For in these individuals resistance to implanted cells need by no means carry with it resistance to a tumor-producing agent.

SUMMARY.

Ultraviolet light rapidly kills the cells of a transplantable sarcoma of the fowl without notably injuring the etiological agent associated therewith. The Roentgen ray has little effect on either cells or agent.

Fowls manifest two sorts of resistance to the avian tumor, one directed against the implanted tumor cells as such, the other against the action of the etiological agent to cause a neoplastic change. In the individual fowl the two resistances appear to be independent of one another, though they may exist together or may both be absent. A recognition of them will perhaps explain some features in the biology of other tumors.

The work has been done with the assistance of Dr. Linda Lange.

EXPLANATION OF PLATE 32.

FIG. 1. A photograph taken eight days after the implantation of material exposed to the Roentgen rays, showing the discrete character of the intradermal tumors. Tumors have arisen, thus far, only from the fresh material. The dried tissue was put in the left pectoral strip. The large mass to the left of the upper end of the sternum is the crop.

FIG. 2. Intradermal tumors in the pectoral strips. The preparation has been turned so that the sternal keel, if present, would lie horizontally along its middle.

The upper line of growths (right pectoral strip) has resulted from the inoculation of fresh tumor tissue exposed in a glass tube to the ultraviolet light for various periods. In the lower line are growths from some of the same material exposed in the same way but in a quartz tube. At the extreme left of each line (left of the photograph) is a tumor from the control material. The periods of irradiation become longer as one proceeds to the right. The photograph was taken on the seventeenth day after the inoculations.

It will be seen that the ultraviolet rays have failed to act through glass, but through quartz their action has been such that at the end of seventeen days tumors are just beginning to appear from the material irradiated longest (fifteen and thirty minutes).

THE EFFECT OF INTRASPINOUS INJECTIONS OF SALVARSAN AND NEOSALVARSAN IN MONKEYS.*

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The use of intraspinous injections of antimeningococcus serum in the treatment of epidemic cerebrospinal meningitis (1), and the increased efficiency of serum so used over serum injected intravenously, has established the value of local therapy in the treatment of this infectious disease of the central nervous system. The desirability of obtaining a method of local application of curative agents in syphilitic infections of the nervous system therefore at once suggests itself.

Horsley (2) in 1910 advocated washing out the cerebrospinal canal, in cases of syphilitic disease, with 1 to 1,000 solutions of bichloride of mercury. This method, however, was soon abandoned. The well known irritating properties of salvarsan when injected into the tissues prohibited the use of this drug in strong concentrations. It was considered desirable, however, to investigate the possibility of the use of minute quantities of salvarsan and neosalvarsan intraspinously, and the intraspinous injection of small quantities of these drugs was therefore performed in monkeys. The results obtained are presented in this communication.

TECHNIQUE.

Macacus rhesus and *Macacus nemestrinus* monkeys were used in the experiments. Under ether anesthesia lumbar puncture was performed and a small quantity of spinal fluid withdrawn. By means of a two cubic centimeter Luer glass syringe, the substance to be tested was then slowly injected through the lumbar puncture needle.

* Received for publication, June 27, 1913.

TABLE I.

No. of animal.	Day of experiment.								Remarks.
	1st.	3d.	4th to 8th.	10th.	12th.	13th to 18th.	20th.	27th.	
Macacus rhesus 1	Cell count 4. Intraspinous injection of 8 mg. salvarsan in 2 c.c. 40% human serum	Cell count 1,100				Cell count 450 (16th day)			Recovered. Died six weeks later of pulmonary tuberculosis. The lumbar and dorsal portions of the cord showed some superficial congestion.
Macacus rhesus 2	Bloody fluid. Intraspinous injection of 3.2 mg. salvarsan in 2 c.c. 40% human serum	Dry tap	Paralysis of legs began on 3d day and became complete	Dry tap					No recovery from paralysis. Killed.
Macacus rhesus 3	Cell count 55. Intraspinous injection of 1.6 mg. salvarsan in 2 c.c. 40% human serum	Cell count 1,180 (2d day)	Cell count 1,600 (5th day)			Cell count 260 (13th day)			Reinjected two months later and died during injection. Death suggested human serum anaphylactic shock. Brain and cord normal.
Macacus rhesus 4	Cell count 6. Intraspinous injection of 1 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 1,280	Cell count 2,000 (8th day)	Died, miliary tuberculosis					Marked subpial congestion of lumbar and dorsal cord; no tubercles seen in brain or cord.

TABLE I—*Continued.*

No. of animal.	Day of experiment.								Remarks.
	1st.	3d.	4th to 8th.	10th.	12th.	13th to 18th.	20th.	27th.	
Macacus rhesus 5	Cell count 3. Intraspinous injection of 0.4 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 890	Cell count 8 (8th day)			Cell count 6. Intraspinous injection of 0.4 mg. salvarsan in 1.6 c.c. 40% monkey serum (18th day)	Cell count 2,510	Cell count 69	Recovered.
Macacus nemestrinus 6	Cell count 90. Intraspinous injection of 0.2 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 855		Cell count 544					Recovered. Died six months later of tuberculosis. Brain and cord normal.
Macacus nemestrinus 7	Cell count 29. Intraspinous injection of 0.1 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 405		Cell count 104		Died (13th day)			Pulmonary tuberculosis, chronic nephritis. Brain and cord normal.
Macacus rhesus 8	Cell count 10. Intraspinous injection of 0.3 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 334		Cell count 66. Intraspinous injection of 1.5 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 4,082				Recovered.

No. of animal.	Day of experiment.								Remarks.
	1st.	3d.	4th to 8th.	10th.	12th.	13th to 18th.	20th.	27th.	
Macacus rhesus 9	Cell count 35. Intraspinous injection of 0.15 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 50		Cell count 36. Died under ether					Brain and cord normal.
Macacus rhesus 10	Cell count 98. Intraspinous injection of 0.045 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 335		Cell count 60. Intraspinous injection of 0.15 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 84				Recovered.
Macacus rhesus 11	Cell count 122. Intraspinous injection of 0.45 mg. neosalvarsan in 1.6 c.c. 0.9% sodium chloride	Cell count 184							Recovered.
Macacus rhesus 12	Cell count 14. Intraspinous injection of 0.9 mg. neosalvarsan in 1.6 c.c. 0.9% sodium chloride	Cell count 351							Recovered. Died two months later of miliary tuberculosis. Brain and cord normal.

The salvarsan solutions used consisted of one cubic centimeter of the alkaline solution of the drug in 0.9 per cent. sodium chloride, and 0.6 of a cubic centimeter of human or normal monkey serum, giving a dilution of serum to 40 per cent. Solutions of neosalvarsan in 0.9 per cent. sodium chloride were similarly injected in 40 per cent. monkey serum. In two monkeys injected with neosalvarsan, the serum was omitted and a simple solution of the drug in 0.9 per cent. sodium chloride was used. In three monkeys second injections were also made.

In judging the irritating properties of these various substances, the cell count in the spinal fluid was used as indicator. A count of the cells was made before treatment, two days after treatment, and again about a week later. The cell counts were made in the usual manner with a white blood-counting pipette and a Thoma-Zeiss chamber. The results of the experiments are given in table I.

The cell count in the spinal fluid of monkeys, before treatment had been begun, was found to vary within wide limits. In addition to those recorded in the table, four other normal monkeys were punctured to determine this point. Their spinal fluids showed cell counts of 10, 40, 85, and 137 per cubic millimeter. Apparently healthy monkeys, therefore, yielded fluids showing counts varying from 3 to 137 per cubic millimeter. This is in striking contrast to man, where the normal count varies from 0 to 5 per cubic millimeter, and in healthy individuals probably never exceeds the latter figure. The cell count following intraspinous injections also varied widely. The highest obtained was 4,082 per cubic millimeter (*Macacus rhesus* 8). This followed the injection of 1.5 milligrams of neosalvarsan, nine days after 0.3 of a milligram had been injected.

Reference to table I shows that the injection of even small quantities of salvarsan is very irritating. 3.2 milligrams caused in one monkey (*Macacus rhesus* 2) paralysis of the hind legs persisting for some months. The injection of one milligram of salvarsan and over produced in every case (*Macacus rhesus* 1, 3, and 4) cell counts of over 1,000 per cubic millimeter, and the injection of even 0.2 of a milligram produced quite a profound cellular reaction which still persisted ten days after the injection (*Macacus nemestrinus* 6).

0.1 of a milligram of salvarsan produced a much less severe reaction (*Macacus nemestrinus* 7).

The injections of neosalvarsan were, on the whole, less irritating, but *Macacus rhesus* 8 showed a severe reaction,—4,082 cells per cubic millimeter on the second injection.

One striking result was the apparent increase in the irritation produced by a second injection in another monkey (*Macacus rhesus* 5). 0.4 of a milligram of salvarsan produced on first injection a cell count of 890 per cubic millimeter; on reinjection, eighteen days later, the same amount of salvarsan was followed by a count of 2,570 cells per cubic millimeter. The increase in cells in these fluids following injections of salvarsan and neosalvarsan consisted almost entirely of polynuclear leucocytes.

Wechselmann (3) studied the effect of salvarsan when injected directly into the brains of dogs and rabbits. Injections of 0.5 to 1 c.c. of a concentration of 1:500 and 1:1,000, that is, of 1 mg. of salvarsan, caused convulsions, weakness of the limbs, and death in from two to four days. Concentrations, however, of 1:2,000 and 1:4,000, that is, 0.5 to 0.25 mg. of salvarsan, produced no disturbance.

In 1912 we reported on the injection of minute quantities of neosalvarsan intraspinously in a case of tabes dorsalis (4). The severe lightning pains and temporary retention of urine which sometimes followed this procedure caused us to discontinue its use.

Wechselmann (5) has subsequently reported the results of intraspinous injections of neosalvarsan in four patients. One paretic was given 3 mg. of neosalvarsan intraspinously and two weeks later 1 mg. There was no reaction. Another paretic was given 0.5 mg. Two children with congenital syphilis were also injected with from 1 to 1.5 mg. without any reaction occurring.

More recently Marinesco (6) has reported severe results from the use of neosalvarsan intraspinously. Thirteen patients were injected, and in three the treatment was repeated. He used, as a rule, 4 c.c. of a 1:800 solution or about 5 mg. of neosalvarsan. While in one or two patients no reaction occurred, the reaction in the majority was severe, and in several cases more serious effects were observed. Thus, in eight patients there was incontinence of urine or else retention, requiring constant catheterization. In three cases there was weakness of the extremities, and in one anesthesia of the rectum, legs, and buttocks.

In a previous communication (7) we have described the use of intraspinous injections of salvarsanized serum in the treatment of syphilitic infections of the central nervous system. The method is to bleed patients one hour after an intravenous injection of salvarsan, separate the serum, and reinject this serum into the sub-

arachnoid space. In view of the favorable effects of the injections of this serum and the risk of causing injury by the direct injection of neosalvarsan, it seems advisable to confine our efforts at the local therapy of syphilis of the central nervous system with salvarsan to the use of such salvarsanized serum.

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A STUDY OF THE SPIROCHÆTICIDAL ACTION OF THE SERUM OF PATIENTS TREATED WITH SALVARSAN.*

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While studying, during the past two years,[1] the effects of the intraspinous mode of treatment of syphilis of the central nervous system, we have incidentally tested the effects of salvarsanized blood serum upon the spirochæta of relapsing fever.

Gonder[2] noted that the serum of rats injected intravenously with large doses of salvarsan exhibited a spirochæticidal effect on *Spirochæta recurrentis.* The rat serum was permitted to act upon a suspension of spirochætæ which were subsequently injected intraperitoneally into mice; the mice remained free of infection, while the controls injected with spirochætæ acted on by normal rat serum developed infection. *Spirochætæ gallinarum* were similarly injured by the blood serum of hens intravenously injected with salvarsan, while intramuscular injections were less active. The spirochæticidal effect was observed only after the treated spirochætæ were injected into animals, as no difference in motility between treated and untreated spirochætæ could be demonstrated. The blood taken shortly after the salvarsan injection was most active, and the spirochæticidal effect disappeared in a few hours, but was parallel with the intensity of the P-dimethylamidobenzaldehyde reaction of the serum for salvarsan. Finally, the serum of salvarsan-treated rats exhibited a curative action in mice infected with *Trypanosoma brucei.*

METHODS.

In the present study *Spirochæta duttoni,* propagated in white rats or mice, has been employed. On the third day after inoculation, the blood usually contained fifty or more spirochætæ per field of the dark-field microscope. When the virulence of the spirochætæ fell off, it was raised by more frequent animal passage. The suspension for performing a test was prepared by bleeding an infected mouse or rat into normal saline, and the blood was diluted

* Received for publication, June 27, 1913.

[1] Swift, H. F., and Ellis, A. W. M., *New York Med. Jour.,* 1912, xcvi, 53.

[2] Gonder, R., *Ztschr. f. Immunitätsforsch., Orig.,* 1912, xv, 257.

with saline until the required number of spirochætæ per field was obtained. One cubic centimeter of the spirochæta suspension was mixed with one cubic centimeter of the serum or dilution of serum, as indicated in the protocols, the resulting mixture being well shaken and then incubated at 37° C. for one hour. One cubic centimeter of the mixture, representing 0.5 of a cubic centimeter of the spirochæta suspension, was injected into mice intraperitoneally. The blood of the inoculated mice was examined daily with the dark-field microscope and twenty fields of each preparation were counted. The average number of spirochætæ per field is indicated in the protocols. Control mice inoculated with the same amount of untreated spirochætæ or spirochætæ mixed with untreated serum were used. Only those experiments in which the controls showed a good infection were accepted.

PROTOCOL I.

The Action of the Serum of a Normal Rabbit Treated Intravenously with Neosalvarsan.

Mouse.	Rabbit serum obtained after treatment.	Dilution of serum.	Day after inoculation.				
			1	2	3	4	5
B—1	Immediately	1 : 1	0	0	0	0	0
B—2	Immediately	1 : 2	0	0	0	0	0
B—3	Immediately	1 : 4	0	±	+	0	0
C—1	1 hour	1 : 1	0	0	0	0	0
C—2	1 hour	1 : 2	0	± w	++	+++	0
C—3	1 hour	1 : 4	+	+++	+++	0	0
D—1	2 hours	1 : 1	± w	±	0	0	0
D—2	2 hours	1 : 2	+	+++	++	0	0
D—3	2 hours	1 : 4	++	+++	+	0	0
E—1	4 hours	1 : 1	± w	+	+	0	0
E—2	4 hours	1 : 2	+	++	0	0	0
E—3	4 hours	1 : 4	++	+++	+++	0	0
F—1	6½ hours	1 : 1	+	++	±	0	0
F—2	6½ hours	1 : 2	++	+++	+	0	0
F—3	6½ hours	1 : 4	++	++++	++++	0	0
Control							
A—1	Before	1 : 1	++	++++	++++	0	0

± w = between 1 and 19 spirochætæ in twenty fields.
± = 1 per field.
+ = 2 to 10 per field.
++ = 11 to 20 per field.
+++ = 20 to 50 per field.
++++ = more than 50 per field; countless.

A normal rabbit was bled into centrifuge tubes before and at intervals after intravenous injections of a watery solution of neosalvarsan in the dose of 0.15 gm. (equivalent to 0.1 gm. salvarsan) per kilogram of body weight. The serum was allowed to separate over night and centrifugalized the next day. The spirochætal blood came from a rat inoculated three days previously; it was diluted to 20 c.c. with saline, so that each field contained between fifteen and eighteen spirochætæ. This suspension was mixed with various dilutions of the rabbit serum, incubated one hour and injected into mice.

The spirochætæ from each tube showed no diminution of motility under the dark-field microscope. The rabbit serum had a definite spirochæticidal effect, which was most marked in that taken immediately after the neosalvarsan injection and steadily diminished as the interval between treatment and bleeding increased, until it had nearly disappeared at the end of six and one half hours. The effect also disappeared in higher dilutions of the serum. Hence the action of the rabbit serum accords with the results obtained by Gonder.

PROTOCOL II.

The Action of the Heated Serum of a Rabbit Treated Intravenously with Neosalvarsan.

Mouse.	Rabbit serum obtained after treatment.	Dilution of serum.	Serum heated or unheated.	Day after inoculation.			
				1	2	3	4
B—1	Immediately	1 : 1	Unheated	0	0	0	0
B—2	Immediately	1 : 2	Unheated	0	0	0	0
B—3	Immediately	1 : 4	Unheated	0	+	0	0
B—4	Immediately	1 : 1	Heated	0	0	0	0
B—5	Immediately	1 : 2	Heated	0	0	0	0
B—6	Immediately	1 : 4	Heated	0	0	0	0
C—1	1 hour	1 : 1	Unheated	0	± w	0	± w
C—2	1 hour	1 : 2	Unheated	0	±	+	0
C—3	1 hour	1 : 4	Unheated	± w	+++	0	0
C—4	1 hour	1 : 1	Heated	0	0	0	0
C—5	1 hour	1 : 2	Heated	0	0	0	0
C—6	1 hour	1 : 4	Heated	0	0	0	0
D—1	2 hours	1 : 1	Unheated	0	+	± w	0
D—2	2 hours	1 : 2	Unheated	±	++	+	0
D—3	2 hours	1 : 4	Unheated	+	+++	+	0
D—4	2 hours	1 : 1	Heated	0	0	0	0
D—5	2 hours	1 : 2	Heated	0	0	0	0
D—6	2 hours	1 : 4	Heated	0	0	0	0
Controls							
A—1	Before	1 : 1	Unheated	++	++++	0	0
A—4	Before	1 : 1	Heated	++	++++	++	0
X—1	—	—	—	++	++++	++	0

A normal rabbit was injected intravenously with neosalvarsan in the dose of 0.15 gm. (=0.1 gm. salvarsan) per kilogram of body weight; it was bled before, immediately after, and one and two hours after treatment. The separated serum was divided into two lots and one half of each lot was heated at 56° C. for thirty minutes. Various dilutions of the unheated and heated sera were incubated with a suspension of *S. duttoni* and injected into mice.

The human serum, injected intraspinously into patients with syphilis of the central nervous system, is heated to 56° C. for one half hour, in order to kill any microörganisms which might be present. After observing that the serum of neosalvarsan-treated rabbits exerted a spirochæticidal action, we tested the effect of heating the serum upon its action on the spirochæta. Protocol II shows that instead of decreasing the spirochæticidal action, the heating markedly increased it. The mice inoculated with spirochætæ, treated with rabbit serum which was withdrawn one and two hours after the neosalvarsan injection, all developed some, but a less degree of, infection than the control animals; while the corresponding mice, injected with spirochætæ acted upon by heated serum, all remained free from infection. Moreover, the heated serum, taken two hours after the neosalvarsan injection, exerted as strong spirochæticidal action as did the unheated serum taken immediately after the injection, while the serum obtained from the rabbit before treatment (serum A) was devoid of action whether unheated or heated. Hence, the effect of heating was to increase the spirochæticidal action of the salvarsanized serum, and not to render more effective some spirochæticidal substance in normal serum. The serum of rabbits that received 0.075 of a gram of neosalvarsan per kilogram of body weight was diluted 1 to 5 and 1 to 10, as well as 1 to 2 and 1 to 4, and acted in the same manner.

PROTOCOL III.

Comparison of the Prophylactic and Curative Action of the Serum of a Neosalvarsan-Treated Rabbit.

Mouse.	Rabbit serum obtained after treatment.	Heated or unheated.	Serum injected.	Treatment at time of inoculation	Day after inoculation. 1	2	3	4	5
B—1	Immediately	Unheated	Intraperitoneally		0	0	0	0	0
B—2	Immediately	Heated	Intraperitoneally		0	0	0	0	± w
C—1	1 hour	Unheated	Intraperitoneally		0	+	++	± w	± w
C—2	1 hour	Heated	Intraperitoneally		0	0	0	0	± w

Mouse.	Rabbit serum obtained after treatment.	Heated or unheated.	Serum injected.	Day after inoculation.					
				1		2	3	4	5
Controls									
A—2	Before	Heated	Intraperitoneally	+		++	++	0	0
X	—	—	—	±		++	++	0	0
B—5	Immediately	Unheated	Intraperitoneally	± ± ±	Treatment on day following inoculation	++	++	0	0
B—6	Immediately	Heated	Intraperitoneally			+	0	0	0
C—5	1 hour	Unheated	Intraperitoneally			++	++	0	0
C—6	1 hour	Heated	Intraperitoneally			+	++	0	0

A normal rabbit was injected intravenously with neosalvarsan, 0.075 gm. (= 0.05 gm. salvarsan) per kilogram of body weight, and bled before, immediately after, and one hour after treatment. The serum was divided and one half of each specimen was heated at 56° C. for thirty minutes. Mice of the same weight were each inoculated intraperitoneally with 0.5 c.c. of a suspension of infected mouse blood, so diluted that there were five to six spirochætæ per field. Part of the mice were immediately injected with 0.5 c.c. of various specimens of heated and unheated treated rabbit serum. The remainder were treated the following day, when all had developed a moderate infection; they received 0.5 c.c. of the same serum.

The prophylactic effect of the rabbit serum was more marked than the curative action. Since the spirochætæ and serum were not brought into contact in the test-tube, as in the previous experiments, the action of the serum was exerted *in vivo*. The increased action of the heated serum was again brought out, both as regards the protective and curative effects. In fact, no curative action whatever was exhibited by the unheated serum.

Having determined that the serum of rabbits injected intravenously with neosalvarsan exercised both prophylactic and curative action, the study was continued with human serum which had been used in the intraspinous treatment of patients with syphilis of the central nervous system.

A patient, weighing 57 kilograms, with tabes dorsalis, received 0.9 gm. neosalvarsan intravenously. The amount per kilogram of body weight was practically 0.015 neosalvarsan (= 0.01 salvarsan). Blood was taken before, immediately after, and one hour after treatment. The serum was separated and half of each specimen was heated at 56° C. for thirty minutes. The spirochæta suspension consisted of the blood of two mice on the third day of infection, diluted to 20 c.c. 1 c.c. of each of the various dilutions of serum was mixed with 1 c.c. of the spirochæta suspension and incubated one hour at

PROTOCOL IV.

The Action of the Serum of a Patient Treated Intravenously with Neosalvarsan.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Remarks.	Day after inoculation.									
				1	2	3	4	5	6	7	8	9	10
B—1	Immediately	1 : 1	Unheated	± w	++	+++	0	0	0	0	0	0	0
B—2	Immediately	1 : 2	Unheated	± w	++	+++	0	0	0	0	± w	± w	0
B—3	Immediately	1 : 4	Unheated	± w	++	Dead	—	—	—	—	—	—	—
B—4	Immediately	1 : 1	Heated	0	0	0	0	0	0	0	0	0	0
B—5	Immediately	1 : 2	Heated	0	0	0	0	0	+	± w	0	0	0
B—6	Immediately	1 : 4	Heated	0	0	0	0	0	± w	±	0	0	0
C—1	1 hour	1 : 1	Unheated	0	0	++	± w	± w	Dead	—	—	—	—
C—2	1 hour	1 : 2	Unheated	± w	+	+++	Dead	—	—	—	—	—	—
C—3	1 hour	1 : 4	Unheated	± w	++	+++	0	0	0	0	0	0	± w
C—4	1 hour	1 : 1	Heated	0	0	0	0	0	0	0	0	0	0
C—5	1 hour	1 : 2	Heated	0	0	0	0	0	± w	± w	0	± w	± w
C—6	1 hour	1 : 4	Heated	± w	+	++++	0	0	0	0	0	0	0
C—7	1 hour	1 : 1	Centrifugate of C—4	0	0	0	0	0	0	0	0	0	0
C—8	1 hour	1 : 2	Centrifugate of C—5	0	0	0	0	0	0	± w	0	0	0
C—9	1 hour	1 : 4	Centrifugate of C—6	±	+	++++	0	0	± w	± w	+	0	0
Controls													
A—1	Before	1 : 1	Unheated	+	+++	++++	++++	Dead	—	—	—	—	—
A—4	Before	1 : 1	Heated	+	+++	+++	++	0	0	0	± w	±	+
X	—	—	—	+	+++	+++	0	0	0	0	± w	±	0

37°. Mice were then injected intraperitoneally with 1 c.c. of the mixture. To determine whether the serum acted inside or outside the animal body, the mixtures of serum and spirochætæ, C–4, C–5, and C–6, after incubation, were centrifugalized for twenty-five minutes and all the spirochætæ thrown down, as shown by microscopical examination of the supernatant fluid. This supernatant fluid was pipetted off and the spirochætæ resuspended in normal saline and injected intraperitoneally.

The spirochæticidal action of the unheated human serum obtained immediately after and one hour after treatment was practically identical. The serum obtained immediately after treatment and heated was, however, more active than the heated specimen obtained an hour later. The heating increased the action of the human serum in the same manner as it increased that of rabbit serum. The spirochætæ acted on by the serum *in vitro* and centrifugalized free from serum produced infection in mice only in the same degree as did the corresponding dilutions in which both serum and spirochætæ were injected. From this it follows that the spirochæticidal action took place in the test-tube, though this action could be brought out only after the treated spirochætæ were injected into susceptible animals, since the organisms were actively motile after their treatment. Castelli[3] in a similar experiment noted that dilute solutions of neosalvarsan in normal serum exerted in test-tubes a spirochæticidal action upon *Spirochæta recurrentis* and *Spirochæta gallinarum,* and the effect was demonstrable only after the microörganisms were injected into animals. He concluded that the salvarsan was attached directly to the protoplasm of the parasites, and that the parasiticidal action was probably completed by the body cells.

PROTOCOL V.

The Duration of the Spirochæticidal Action of the Serum of a Neosalvarsan-Treated Patient.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Day after inoculation.			
			1	2	3	4
C—1	1 hour	1 : 1	0	0	0	0
C—2	1 hour	1 : 2	0	0	0	0
C—3	1 hour	1 : 4	0	± w	+	0

[3] Castelli, G., *Ztschr. f. Chemotherap., Orig.,* 1912, i, 122.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Day after inoculation.			
			1	2	3	4
D—1	3 hours	1 : 1	0	0	0	0
D—2	3 hours	1 : 2	0	0	0	0
D—3	3 hours	1 : 4	± w	±	++	0
E—1	6 hours	1 : 1	0	0	0	0
E—2	6 hours	1 : 2	0	0	+	0
E—3	6 hours	1 : 4	±	+	0	0
F—1	11 hours	1 : 1	0	± w	+	0
F—2	11 hours	1 : 2	± w	+	+	0
F—3	11 hours	1 : 4	+	+	0	Dead
G—1	24 hours	1 : 1	±	+++	++	0
G—2	24 hours	1 : 2	±	+++	+++	0
G—3	24 hours	1 : 4	+	+++	++++	0
Controls						
A	Before	1 : 1	+	+++	++	0
X	—	—	± w	+++	±	0

Some weeks later the patient used in protocol IV again received 0.9 gm. of neosalvarsan intravenously. Blood was taken before, and one, three, six, eleven, and twenty-four hours after treatment. The serum was separated and heated at 56° C. for thirty minutes. Various dilutions of each bleeding were allowed to act on the spirochætæ, and the mixture injected into mice, as in the previous experiments.

The spirochæticidal action of the heated serum decreased in proportion to the time that elapsed between treatment and bleeding, some spirochæticidal action being still present eleven hours after treatment, while after twenty-four hours the serum showed no more effect than normal serum. The Abelin[4] diazo reaction for salvarsan was applied to the serum used in this experiment. One cubic centimeter of each specimen was tested. A deep rose red ring is the typical reaction. The results are shown in protocol V–A.

PROTOCOL V–A.

The Abelin Diazo Reaction in the Serum Used in Protocol V.

Serum.	Obtained after treatment.	Reaction.
C	1 hour	Deep red ring.
D	3 hours	Moderate red ring.
E	6 hours	Orange red ring.
F	11 hours	Light orange ring.
G	24 hours	Green brown ring.
A	Before	Green brown ring.

[4] Abelin, J., *München. med. Wchnschr.*, 1912, lix, 81.

The color reaction decreased in proportion with the spirochæticidal action of the serum. A slight color reaction was still demonstrable in the eleven hour serum, while the twenty-four hour specimen and serum before treatment reacted alike. In another experiment in which the spirochæticidal action of the serum was slightly less marked, the diazo color reaction and the spirochæticidal action were also parallel. Hence, the experiments suggest that the action of the serum on the spirochæta is due to its salvarsan content.

PROTOCOL VI.

Comparison of Action of Serum of Patients Treated with Salvarsan and Neosalvarsan.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Heated or unheated.	Day after inoculation. 1	2	3	4	5	6
B—1	10 minutes	1 : 1	Heated	0	± w	±	0	0	0
B—2	10 minutes	1 : 2	Heated	± w	++	+++	± w	Dead	—
B—3	10 minutes	1 : 4	Heated	+	+++	+++	0	0	0
B—4	10 minutes	1 : 1	Unheated	± w	+	++	± w	± w	0
C—1	1 hour	1 : 1	Heated	± w	+	±	0	0	0
C—2	1 hour	1 : 2	Heated	±	+	++++	± w	0	0
C—3	1 hour	1 : 4	Heated	+	++	++++	++	± w	++
C—4	1 hour	1 : 1	Unheated	0	+	+	0	0	0

Neosalvarsan Serum.

BB—1	10 minutes	1 : 1	Heated	0	0	0	0	0	0
BB—2	10 minutes	1 : 2	Heated	± w	+	++	0	0	0
BB—3	10 minutes	1 : 4	Heated	+	++	++	0	0	0
BB—4	10 minutes	1 : 1	Unheated	0	0	0	0	0	0
CC—1	1 hour	1 : 1	Heated	0	+	++	0	0	0
CC—2	1 hour	1 : 2	Heated	± w	++	+	0	0	—
CC—3	1 hour	1 : 4	Heated	±	+++	++	0	0	—
CC—4	1 hour	1 : 1	Unheated	± w	++	+++	+++	± w	—
Controls									
A—1	Before	1 : 1	Unheated	±	++	++++	+	0	0
AA—1	Before	1 : 1	Unheated	+	+++	++++	++	Dead	—
X	—	—	—	+	+++	+++	Dead	—	—

A patient weighing 67 kilograms, with latent secondary syphilis, received 0.5 gm. salvarsan, and another weighing 57 kilograms, with tabes dorsalis, received 0.75 gm. neosalvarsan (corresponding to 0.5 gm. salvarsan) intravenously. The dose of salvarsan per kilogram for the first was 0.0075 gm., and for the second 0.0088 gm. Blood from each was taken before, ten minutes after, and one hour after treatment. Half of each specimen of the serum was heated at 56° C. for thirty minutes. The experiments were as before.

The results indicate that the serum of patients treated with neosalvarsan exerts slightly more spirochæticidal action on *Spirochæta duttoni* than that of patients treated with salvarsan. This difference may be due to the fact that neosalvarsan is more soluble than salvarsan. When the alkaline salvarsan is brought into contact with the blood, a part is precipitated and this precipitate is probably deposited in the organs. The neosalvarsan, being neutral, is not precipitated, and hence more of it probably remains in the blood for a short period after the intravenous injection. To test this point, two sera were submitted to the Abelin diazo reaction for salvarsan. Various dilutions, ranging between whole serum and 1 to 32 were prepared and one cubic centimeter of each dilution was tested.

PROTOCOL VI–A.

The Abelin Diazo Reaction in the Sera Used in Protocol VI.

Dilution of serum.	Salvarsan serum. Serum B.	Neosalvarsan serum. Serum BB.
1 : 1	Orange red ring	Deep red ring.
1 : 2	Light orange red ring	Orange red ring.
1 : 4	Red brown ring	Light orange red ring.
1 : 8	Faint red brown ring	Red brown ring.
1 : 16	Green brown ring	Green brown ring.
1 : 32	Green brown ring	Green brown ring.
	Serum C	Serum CC.
1 : 1	Orange red ring	Deep red ring.
1 : 2	Light orange red ring	Light orange red ring.
1 : 4	Faint red brown ring	Red brown ring.
1 : 8	Green brown ring	Green brown ring.
1 : 16	Green brown ring	Green brown ring.
1 : 32	Green brown ring	Green brown ring.
	Serum A	Water.
1 : 1	Brown ring	Green brown ring.

The results indicate that more salvarsan was present in the serum of the patient treated with neosalvarsan than in the one treated with salvarsan. The difference was more marked in the blood drawn ten minutes after treatment than one hour later. The poorer salvarsan content probably explains the smaller spirochæticidal action in the latter serum. This difference need not necessarily apply to *Treponema pallidum,* for Castelli[5] showed that while the curative dose of salvarsan and neosalvarsan, computed in

[5] Castelli, G., *loc. cit.,* p. 321.

weight of salvarsan, was the same for *Spirochæta recurrentis* infections in mice, the rule did not hold for the pallidum, for twice as much neosalvarsan as salvarsan was required to cure syphilis in rabbits.

PROTOCOL VII.

The Effect of Adding Normal Serum to Heated Salvarsanized Serum.

Mouse.	Serum B obtained 10 minutes after treatment.	Serum A obtained before treatment.	Normal saline.		Amount of mixture injected.	Day after inoculation.				
						1	2	3	4	6
	Heated			1 c.c. spirochæta suspension added to each tube. Incubation at 37° C. 1 hour.						
B—1	1.0 c.c.	—	—		1.0 c.c.	0	0	0	0	0
B—2	0.5 c.c.	—	0.5 c.c.		1.0 c.c.	0	0	0	0	±w
B—3	0.25 c.c.	—	0.75 c.c.		1.0 c.c.	±w	Dead	—	—	—
		Unheated								
B—4	1.0 c.c.	1.0 c.c.	—		1.5 c.c.	0	±w	±w	+	0
B—5	0.5 c.c.	0.5 c.c.	—		1.0 c.c.	±w	±w	++	±w	0
B—6	0.25 c.c.	0.25 c.c.	0.5 c.c.		1.0 c.c.	±	++	++++	+++	Dead
		Heated								
B—7	1.0 c.c.	1.0 c.c.	—		1.5 c.c.	0	0	0	0	0
B—8	0.5 c.c.	0.5 c.c.	—		1.0 c.c.	0	0	+	0	±
B—9	0.25 c.c.	0.25 c.c.	0.5 c.c.		1.0 c.c.	±w	+	++	Dead	—
	Unheated									
B—10	1.0 c.c.	—	—		1.0 c.c.	±w	++	+++	Dead	—
B—11	0.5 c.c.	—	0.5 c.c.		1.0 c.c.	±w	++	+++	0	0
		Unheated								
B—12	1.0 c.c.	1.0 c.c.	—		1.5 c.c.	±w	+	++	±w	0
B—13	0.5 c.c.	0.5 c.c.	0.5 c.c.		1.0 c.c.	±w	++	++++	+	Dead
Controls										
A—1	—	1.0 c.c.	—		1.0 c.c.	±w	+	+++	+	0
X	—	—	1.0 c.c.		1.0 c.c.	+	+++	++++	++++	Dead

A patient, weighing 57 kilograms, with tabes dorsalis, received 0.75 gm. of neosalvarsan (corresponding to 0.5 gm. of salvarsan) intravenously. Blood was obtained immediately before treatment (serum A) and ten minutes after treatment (serum B). The serum was separated and one half of each specimen heated at 56° C. for one half hour. The suspension of spirochætæ was similar to that of previous experiments. Several sets of tubes were set up. In the first the spirochætæ were mixed with heated serum B, in the second with heated serum B and unheated serum A, in the third with heated serum B and heated serum A, in the fourth with unheated serum B, and in the fifth with unheated serum B and unheated serum A. After incubation for one hour, the various mixtures were injected into mice, each mouse receiving an amount which contained 0.5 c.c. of the original spirochæta suspension.

The spirochæticidal action of the salvarsan-containing serum, increased by heating, was partly abolished by the addition of unheated

normal serum. The addition of heated normal serum to heated salvarsanized serum did not diminish appreciably the action of the salvarsanized serum. The addition of unheated normal serum to unheated salvarsanized serum did not increase its spirochæticidal action. These results indicate that the increased spirochæticidal action of heated salvarsanized serum, as compared with the same serum unheated, is due, in part at least, to the destruction of some inhibiting substance which is contained in both normal and salvarsanized serum. It is probable that the increased activity of heated serum is not due entirely to the destruction of this inhibiting substance, since, if this were the case, the addition of an equal amount of unheated normal serum should supply sufficient inhibiting substance to neutralize the effect produced by heating; the mixture would, therefore, behave exactly like the unheated salvarsanized serum. This, however, was not the case, for the mixture of heated salvarsanized serum and unheated normal serum was always more spirochæticidal than the unheated salvarsanized serum alone. Besides, protocol III, in which both the prophylactic and curative effects of the salvarsanized rabbit serum were exerted within the bodies of mice, indicates that heating increases the action of the serum. If the increased activity were entirely due to the destruction of some inhibiting substance by heating, it would seem that the mouse serum should supply the inhibiting substance, and both unheated and heated serum act alike. The heated serum, however, always excited a more marked action than the unheated serum. It is probable, therefore, that the effect of heating, besides destroying some inhibiting substance in normal serum, increases the spirochæticidal action of the salvarsan-serum mixture by producing some change in the mixture.

PROTOCOL VIII.

The Effect of Adding Cerebrospinal Fluid to Heated Salvarsanized Serum.

Mouse.	Serum C obtained one hour after treatment.	Cerebrospinal fluid S.	Serum A obtained before treatment.	Normal saline.		Amount of mixture injected.	Day after inoculation. 1	2	3	4	5	6	7	8
	Heated				1 c.c. spirochæta suspension in each tube. Incubation at 37°C. 1 hour.									
C—1	1.0 c.c.	—	—	—		1.0 c.c.	0	0	0	0	0	0	0	± w
C—2	0.5 c.c.	—	—	0.5 c.c.		1.0 c.c.	0	0	0	0	0	0	0	0
C—3	0.25 c.c.	—	—	0.75 c.c.		1.0 c.c.	± w	+	++	± w	± w	0	0	0
		Unheated												
C—4	1.0 c.c.	1.0 c.c.	—	—		1.5 c.c.	0	0	0	0	0	0	± w	±
C—5	0.5 c.c.	0.5 c.c.	—	—		1.0 c.c.	0	0	0	0	0	± w	+	± w
C—6	0.25 c.c.	0.25 c.c.	—	0.5 c.c.		1.0 c.c.	± w	+	± w	0	0	0	0	± w
		Heated												
C—7	1.0 c.c.	1.0 c.c.	—	—		1.5 c.c.	0	0	0	0	0	0	0	± w
C—8	0.5 c.c.	0.5 c.c.	—	—		1.0 c.c.	0	0	0	0	± w	+	0	0
C—9	0.25 c.c.	0.25 c.c.	—	0.5 c.c.		1.0 c.c.	± w	± w	++	0	0	0	± w	±
			Unheated											
C—10	1.0 c.c.	—	1.0 c.c.	—		1.5 c.c.	0	0	0	± w	Dead	—	—	—
C—11	0.5 c.c.	—	0.5 c.c.	—		1.0 c.c.	± w	± w	+	0	0	—	± w	0
C—12	0.25 c.c.	—	0.25 c.c.	0.5 c.c.		1.0 c.c.	± w	+	+	0	0	0	± w	± w
			Heated											
C—13	1.0 c.c.	—	1.0 c.c.	—		1.5 c.c.	0	0	0	0	0	± w	+	± w
C—14	0.5 c.c.	—	0.5 c.c.	—		1.0 c.c.	0	0	0	0	0	0	± w	± w
C—15	0.25 c.c.	—	0.25 c.c.	0.5 c.c.		1.0 c.c.	± w	+	0	0	0	0	0	0
	Unheated													
C—16	1.0 c.c.	—	—	—		1.0 c.c.	0	0	0	0	±	++	++	+
C—17	0.5 c.c.	—	—	0.5 c.c.		1.0 c.c.	± w	±	+++	+	0	0	0	± w
C—18	0.25 c.c.	—	—	0.75 c.c.		1.0 c.c.	±	++	+	0	0	0	0	0
Controls														
		Unheated												
S—1	—	1.0 c.c.	—	—		1.0 c.c.	±	+	++	0	0	0	± w	0
		Heated												
S—2	—	1.0 c.c.	—	—		1.0 c.c.	±	++	0	0	0	0	0	0
A	—	—	1.0 c.c.	—		1.0 c.c.	±	+	++++	++	0	0	0	0
X	—	—	—	1.0 c.c.		1.0 c.c.	+	++	++	+	0	0	0	0

A patient, weighing 56 kilograms, with tabes dorsalis, received 0.5 gm. of salvarsan intravenously. Blood was obtained before treatment (serum A) and one hour after (serum C). Twenty-four hours later cerebrospinal fluid was obtained from the same patient. This fluid contained an excess of globulin. Half of each specimen of the serum and half of the cerebrospinal fluid were heated at 56° C. for thirty minutes. An experiment was made in the same manner as the preceding one, with the addition of two sets of tubes, viz., heated salvarsanized serum with unheated cerebrospinal fluid, and heated salvarsanized serum with heated cerebrospinal fluid.

The object was to determine whether cerebrospinal fluid contained an inhibiting substance similar to that in unheated serum. It was necessary to duplicate protocol VII as a control. The addition of normal unheated serum decreased somewhat the spirochæticidal action of the heated salvarsanized serum, as in the preceding experiment. The addition of heated normal serum and of cerebrospinal fluid, both heated and unheated, to the heated salvarsanized serum had only slight effect, shown by delayed infections in the mice receiving mixtures of 0.5 of a cubic centimeter. All the mice (except C—2) which failed to show early infection, developed slight, short infections between the fifth and eighth days. We have frequently noticed that the late developing infections are usually slight and irregular in time of development, so that little weight can be placed upon them beyond showing that all spirochætæ were not killed. The apparent spirochæticidal action of the cerebrospinal fluid alone is probably due to the fact that very little serum was present in the mixture during the period of incubation. We have repeatedly noticed that spirochætæ incubated for one hour in normal salt solution failed to produce as severe an infection as organisms after incubation in normal serum. Under the dark-field microscope, at the end of one hour's incubation, many of the spirochætæ incubated with salt solution appeared granular and had lost their motility, while those incubated with normal serum were actively motile. As cerebrospinal fluid is hypotonic, it is probable that many of the spirochætæ incubated in it were permanently injured. When serum was present in the mixture, the hypotonicity was diminished; hence the spirochætæ were injured less. The inhibitory substance present in normal serum and destroyed by heating at 56° C. is absent from cerebrospinal fluid, even when the fluid contains an abnormal amount of globulin.

In so far as the results obtained with one variety of spirochæta can be applied to another variety, the work here presented suggests that the serum of patients treated with salvarsan should have a distinct spirochæticidal action on *Treponema pallidum*. Probably the best way to treat many of the manifestations of syphilitic infection of the central nervous system is to introduce the therapeutic agent directly into the cerebrospinal fluid, which carries it directly to the diseased tissue. We[6] have been able to demonstrate that the subdural injection of the serum of salvarsan-treated patients has a definite curative action in syphilitic disease of the central nervous system. The above experiments with *Spirochæta duttoni* suggest that this curative action is due to the direct spirochæticidal action of the salvarsanized serum.

SUMMARY.

1. The serum of rabbits treated intravenously with neosalvarsan, and of syphilitic patients treated intravenously with salvarsan or neosalvarsan, has a definite spirochæticidal action upon *Spirochæta duttoni*.

2. Although this spirochæticidal action is exerted *in vitro*, it can be demonstrated only after the treated spirochætæ are injected into susceptible animals.

3. A curative action of the serum of neosalvarsan-treated rabbits is exercised on mice infected with *Spirochæta duttoni*.

4. The spirochæticidal action of the serum of salvarsan-treated rabbits and patients is markedly increased by heating at 56° C. for thirty minutes.

5. The increased spirochæticidal action produced by heating is due in part to the destruction of some inhibitory substance contained in normal serum and in part to a direct effect of the heat upon the serum and salvarsan mixture.

6. Cerebrospinal fluid does not contain the inhibitory substance present in normal unheated serum.

[6] Swift, H. F., and Ellis, A. W. M., *loc. cit.*

AN ELECTROCARDIOGRAPHIC STUDY OF THE ANAPHYLACTIC RABBIT.*

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Plates 33 to 37.

INTRODUCTION.

The subject of cardiac disturbances in serum anaphylaxis has not received much attention from investigators. Gay and Southard,[1] in their extensive histological studies, noted that the heart of the anaphylactic guinea pig often shows hemorrhages; they did not, however, observe any alteration in the functional activity of this organ, as far as we are aware. The first definite observation of a pathological alteration of the heart's activity during serum anaphylaxis was made by Auer and Lewis,[2] who observed that the heart of a guinea pig dying from anaphylaxis showed several abrupt diminutions in rate, and that these rates were in simple ratio to each other. This observation was made by inspection of the exposed heart and by examination of graphic records. The block was thought to be due to asphyxia. This interpretation is in accord with the results Sherrington[3] obtained with decapitated, atropinized cats, where asphyxia produced an abrupt halving of the cardiac rate; it also agrees with the work of Lewis and Mathison,[4] who demonstrated the occurrence of heart-block in cats during asphyxia by means of the electrocardiograph.

During the anaphylactic reaction of the rabbit, one of us[5] has noticed heart-block, both by inspection of the exposed heart and by a graphic method. Further and more detailed evidence will be given in this paper.

Cardiac disturbances during serum anaphylaxis of the cat have been described by Schultz,[6] but his results are difficult to interpret with certainty. In the cat matters are complicated by the fact that horse serum exerts a strongly toxic effect in the unsensitized animal; as little as 0.25 c.c. per kilo usually kills young

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[1] Gay, F. P., and Southard, E. E., *Jour. Med. Research*, 1907, xvi, 146, 176.

[2] Auer, J., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 171; see plate IX, figure 5, for a graphic record.

[3] Sherrington, C. S., *Jour. Physiol.*, 1909, xxxviii, 381.

[4] Lewis, T., and Mathison, G. C., *Heart*, 1910, ii, 47.

[5] Auer, J., *Jour. Exper. Med.*, 1911, xiv, 483 (see plate 44 and plate 47, figure 4).

[6] Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1911–12, iii, 302.

cats, while adult cats are strongly affected but "frequently recover" from this dose.[7] Even 0.1 c.c. per kilo gives a fall in blood pressure both in normal and sensitized cats.[8] The ordinary reinjection dose employed by Schultz seems to have been 0.2 c.c. per kilo. Moreover, Schultz observed traces of blood clot in the hearts of some anaphylactic cats.[9] The cat thus differs strikingly from guinea pigs, rabbits, and dogs in its reaction to horse serum, for the latter animals show no definite toxic effects when comparatively large quantities of not too fresh horse serum are injected intravenously. A normal guinea pig tolerates 5 c.c., a normal rabbit 15 c.c., and a normal dog at least 30 c.c. of horse serum intravenously without any symptoms which even remotely resemble those occurring in anaphylaxis.[10] A consideration of these data forces us to the conclusion that the cardiac disturbances noted by Schultz are not necessarily anaphylactic phenomena.

The anaphylactic dog also shows heart-block and other cardiac disturbances demonstrable by the electrocardiograph. A detailed statement of these changes will be found in another paper.[11]

This brief review of the literature concerning heart disturbances in serum anaphylaxis of the intact animal shows definitely that alterations of the functional activity of this organ are common in this state and apparently form an integral part of anaphylaxis. In the following pages we shall give an account of the main changes which the heart of the anaphylactic rabbit reveals when examined by the electrocardiograph. Details regarding the process of sensitization, which is by no means as certain in the rabbit as in the guinea pig, will be found in a former paper by one of us.

EXPERIMENTAL PART.

The cardiac changes of anaphylactic reaction were studied in twenty-four rabbits. Young animals weighing about one kilo were sensitized by repeated subcutaneous injections of horse serum. About four weeks after the last injection, the animals were given the final, toxic dose of about 9 c.c. into a marginal ear vein, and the cardiac disturbances were studied by means of electrocardiograms taken at short intervals immediately before, during, and after the injection. The records were made with the large Edelmann string galvanometer. The animals were connected with the instrument by electrodes

[7] Schultz, W. H., *loc. cit.*, p. 300.

[8] Schultz, W. H., *loc. cit.*, p. 317.

[9] Schultz, W. H., *loc. cit.*, p. 304.

[10] These doses do not represent the maximal quantities which are tolerated; they are quoted because such doses have been injected in normal animals during the course of other experiments.

[11] Robinson, G. C., and Auer, J., *Zentralbl. f. Physiol.*, 1913, xxvii, 383; *Jour. Exper. Med.*, 1913, xviii, (in press).

usually attached to the right fore leg and left hind leg (the second lead). The string of the galvanometer was arranged so that a deviation of 1 cm. in the curve represents the passage of 1 millivolt. No ether was given during the experiment, and the slight operative procedures hardly demanded it. In eleven of the sensitized rabbits both vagi were cut before the toxic dose of serum was injected and after tracheotomy had been performed. Horse serum was also injected intravenously into two non-sensitized rabbits, from which electrocardiographic records were obtained in the same way as from the others.

The rate of the heart-beat, the relation of auricular to ventricular contractions, and the form of the electrocardiograms were especially considered in studying the records obtained in these experiments. Definite abnormalities in the heart-beat were observed in twenty-two of the twenty-four experiments in which sensitized rabbits were used, and in ten experiments the anaphylactic reaction was fatal, the rabbits dying in from two to nineteen minutes. The average time of death was seven minutes after the beginning of the intravenous injection. In these experiments it is necessary to separate the cardiac disturbances which resulted primarily from the anaphylactic reaction, from those which occurred as phenomena of the dying heart, and cessation of respiration was therefore used as the index of death. Changes in the heart-beat, which occurred after respiration had ceased, were not considered as phenomena of the anaphylactic reaction, although in some instances the electrocardiograms returned to normal after respiration had ceased, while they had been markedly abnormal before.

The intravenous injection of horse serum into two non-sensitized normal rabbits was followed by no such changes in the electrocardiograms as were seen in the sensitized animals. In one control experiment the records indicated that premature ectopic ventricular beats began to occur occasionally about thirty seconds after the beginning of the injection of horse serum and before it was completed. The ectopic beats became more numerous, and one minute after the beginning of the injection they alternated with the normal beats. These ectopic ventricular contractions gradually became less numerous, and in three and a half minutes after the beginning of the injections the records show that they had ceased to occur. Three days later this rabbit was again attached to the string galvanometer and given an intravenous injection of nine cubic centimeters of

normal saline solution. No disturbances of the cardiac mechanism occurred. In the second control experiment the records show that no cardiac disturbances followed the intravenous injection of horse serum.

A second intravenous injection of horse serum was given to seven sensitized rabbits after the effects of the first injection had passed off. All but one of the rabbits in these experiments had shown marked cardiac disturbances after the first injection. After the second injection no disturbances of the heart-beat or other symptoms occurred except in one experiment in which ectopic ventricular contractions appeared. These animals were thus in the anti-anaphylactic state at this time. The fact that ectopic ventricular contractions occurred after the second injection in one of these animals and also in one of the non-sensitized animals after a single injection of horse serum, indicates that the occurrence of such beats probably cannot be considered as significant when they occur during the anaphylactic reaction.

The condition of the various experiments and the results may be summarized briefly as follows:

No. of experiment.	Result.	Vagi.	Complex changes before cessation of respiration.
2	Fatal	Intact	Marked. Temporary partial block. Slight changes in form.
3	Fatal	Intact	Marked. Temporary partial block. T wave much increased.
5	Fatal	Intact	Marked. Abnormal relation of P and R waves. T wave disappeared.
6	Fatal	Intact	Marked. Abnormal relation of P and R waves. S wave developed.
7	Fatal	Intact	Moderate. Curves poor. T waves disappear. Ectopic beats.
8	Fatal	Intact	Marked. Temporary block twice. Marked S and negative T waves developed.
10	Fatal	Cut	Marked. S wave and negative T wave developed.
17	Fatal	Cut	Marked. P waves disappear. Possible auricular fibrillation.
19	Fatal	Cut	Marked. T partly fused with R waves.
25	Fatal	Cut	Marked. P wave disappears. T close to R waves.
1	Not fatal	Intact	Moderate. P wave disappears. T wave becomes absent.
4	Not fatal	Intact	No definite changes.
9	Not fatal	Intact	Marked. Abnormal relation of P and R waves. T wave disappears.
11	Not fatal	Intact	Marked. Abnormal relation of P and R waves. Large S and negative T waves.

No. of experiment.	Result.	Vagi.	Complex changes before cessation of respiration.
12	Not fatal	Intact	Marked. Abnormal relation of P and R waves. Abnormal R. S develops.
13	Not fatal	Intact	Slight. S wave develops. T wave becomes negative.
14	Not fatal	Intact	Slight. S wave develops. T wave disappears.
18	Not fatal	Cut	Marked. Prominent S wave develops and large T waves.
20	Not fatal	Cut	Marked. Abnormal relation of P and R waves. Ventricular complexes vary.
21	Not fatal	Cut	Marked. R waves broad. T waves negative.
22	Not fatal	Cut	Marked. Broad, deep S wave.
23	Not fatal	Cut	Moderate. S wave develops. T wave disappears.
24	Not fatal	Cut	Marked. Abnormal relation of P and R waves. P wave disappears. Broad S wave.
26	Not fatal	Cut	No definite changes.
15	Control	Intact	No changes except ectopic ventricular beats.
16	Control	Intact	No definite changes.

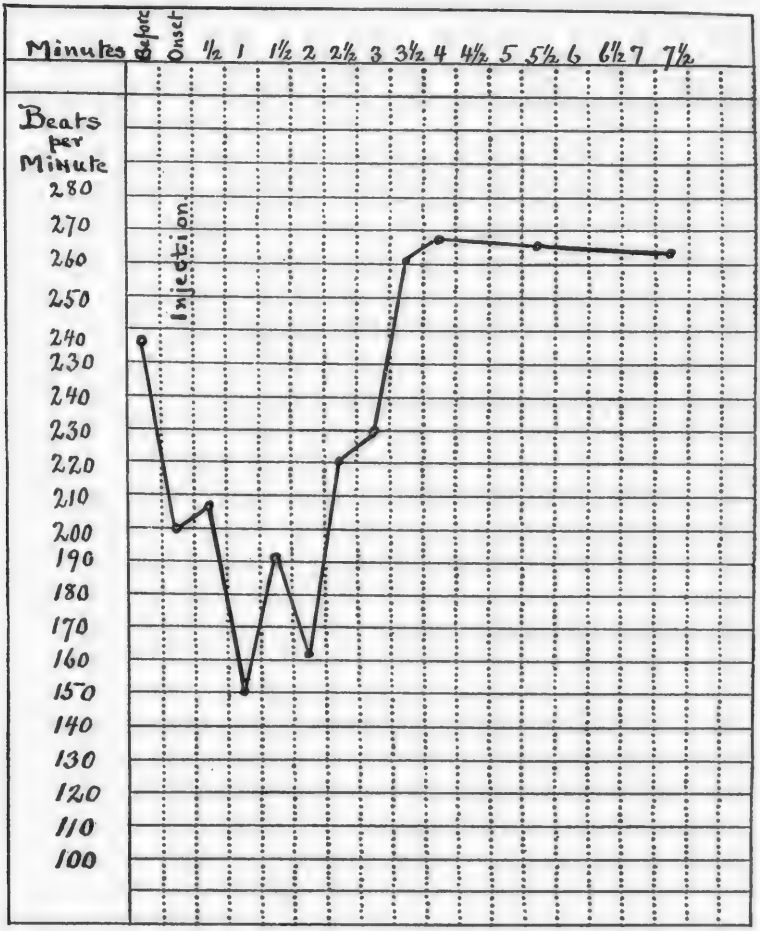
The experiments may be grouped into those in which the anaphylactic reaction was fatal, and those in which the animals recovered. These groups may be subdivided according to whether the vagi were intact or cut.

The changes in cardiac rate may be considered, however, independent of these groups, for seventeen of the twenty-four experiments showed an alteration of rate which bore apparently no relation to the ultimate outcome of the experiments, nor to whether the vagi were intact or not. This change consisted of a slowing of twenty-five beats or more during the first minute after beginning the injection, and then an increase to a rate twenty-five beats or more per minute faster than that present before the injection. This change of rate may be illustrated by a chart constructed from experiment 12, a non-fatal experiment with the vagi intact (text-figure 1). The same initial slowing of the heart is shown in blood pressure records.[12]

The first group consists of six experiments in which the final injection of horse serum was fatal and in which the vagi were intact. In all but one of these cases marked disturbances of the heart-beat developed swiftly, often before the end of the serum injection, and at a time when the respiration was practically undisturbed. The

[12] Auer., J., *Jour. Exper. Med., loc. cit.*, p. 495 (plates 44, 45, and plate 47, figure 4).

most striking disturbance was partial heart-block, which occurred in three of the cases. This condition was always temporary, and the normal sequential beat was established before the changes of the dying heart occurred. In experiment 8 partial heart-block occurred one and a half minutes after the beginning of the injection (figures 1 and 2). The normal sequential beat returned forty-five



TEXT-FIG. 1. Experiment 12. The changes in the cardiac rate in anaphylactic shock.

seconds later with an increase in rate and a moderate change in the form of the complexes (figure 3). Partial heart-block appeared again in one and a quarter minutes, when the complexes had changed markedly in form (figure 4). The block had disappeared five minutes after the beginning of injection (figure 5), but returned again as a phenomenon of the dying heart in less than one minute (figures 6 and 7). In experiment 3 (figure 8) a partial heart-block was present one minute and a half after the injection (figure 9), while in three minutes complete dissociation of auricles and ventricles developed (figure 10). The sequential beat had returned at four minutes after the injection, but with a marked delay in the conduction time (figure 11). Half a minute later partial block had again set in (figure 12). The various electrocardiograms obtained from this animal showed marked changes in form, and the record obtained shortly after the injection shows ventricular complexes very much of the same form as those taken eleven minutes after the injection and one minute after respiration had ceased (figure 13). In each, the sharp, rather prominent T waves are close to the R waves, the down strokes of which are slow and do not reach the base line. This form of ventricular complex seems to be characteristic of the dying heart, as shown by one of us (Robinson).[13] In experiment 2 partial heart-block was present from two to four and a half minutes after the injection and returned again after the normal sequential beat had been present for half a minute, continuing until respiration ceased ten minutes after the injection.

In two experiments an abnormal relation between the P and R waves occurred in the electrocardiograms. This consisted of a temporary shortening of the conduction (P–R) time. Thus in experiment 6 the P–R time was 0.08 of a second before the injection (figure 14), while it became reduced to 0.033 of a second two minutes after the injection was begun (figure 15). In half a minute the P–R time had lengthened to 0.068 of a second. In experiment 5 the P–R time was diminished at forty-five seconds after the onset of the injection, and remained so for one and a quarter minutes. The P–R relation was then normal for one minute, when

[13] Robinson, G. C., *Jour. Exper. Med.*, 1912, xvi, 291.

the P and R waves were again abnormally near each other and partially fused. This same change in the relation of the P and R waves occurred in the electrocardiograms from five other experiments in which the anaphylactic shock was not fatal, three in which the vagi were intact, and two in which they had been cut.

In the four experiments in which a fatal anaphylactic shock occurred after the vagi had been cut, definite abnormalities in the forms of the electrocardiograms appeared in forty-five seconds, one and a half minutes, two minutes, and two and a half minutes respectively after the beginning of the injection. In one experiment respiration ceased at about the same time that the electrocardiograms became abnormal, but in the others intervals of two, four, and eight minutes respectively separated the appearance of abnormalities in the electrocardiograms and the cessation of respiration. The changes in form of the electrocardiograms from these experiments were various. In two the P wave disappeared, and in one of these the electrocardiograms suggest indefinitely that auricular fibrillation was present for a short time. The T waves of ventricular complexes changed definitely in three of the experiments, in two occurring close to or partly fused with the R wave. In the other experiment which showed abnormal T waves, they gradually diminished in size and then became definitely negative or downwardly directed, this change beginning two and a half minutes after beginning the injection and reaching its maximum one and a half minutes later (figures 16 and 17). Thus it is seen that changes occurred in the electrocardiograms from this group of experiments which indicate that both the auricular and the ventricular activity were disturbed, while no definite disturbances of conduction took place.

The experiments in which the anaphylactic shock was not fatal, are conveniently divided into those in which the vagi were intact and those in which the vagi were cut. There were seven experiments with intact vagi, and the electrocardiograms from all but one showed changes which were marked in three, moderate in one, and slight in two. The abnormalities in the electrocardiograms were first seen in from one to three and a half minutes after the beginning of the injection. In all these experiments but one, the electro-

cardiograms returned to the form seen before the injection, the time varying from seven and a half to twenty-one minutes. These changes vary in the different experiments. Those which occurred in experiment 11 are shown in figures 18 to 21; before injection the complexes are well formed, the P–R time is 0.07 and the rate 281; three and a quarter minutes later the R wave is followed by a negative phase, the P–R time is unchanged, while the rate has decreased to 174 beats per minute; fifteen seconds after this a marked arrhythmia with varying electrocardiograms set in, which disappeared in another fifteen seconds, when very abnormal electrocardiograms occurred. This form, consisting mostly of a broad negative wave, persisted until five and a half minutes after the injection, when it began to return gradually to the form seen before the injection.

The T waves were changed in four of these seven experiments, disappearing in three, and in one becoming downwardly directed. The P and R waves had an abnormal relation, temporarily, in three experiments, becoming approximated as in experiment 6 (figures 14 and 15), and in one experiment the P wave disappeared.

Of the seven non-fatal experiments in which the vagi were cut, five showed marked changes in the form of the electrocardiograms; in one there were moderate changes, and in one no changes were seen. The changes appeared after one to five minutes, and the abnormalities lasted from two and a half to five minutes. The changes in this group resemble those which occurred in the non-fatal experiments where the vagi were not cut. In four experiments large, broad, downwardly directed S waves appeared. The T wave changed in three, once increasing in size, once disappearing, and once becoming downwardly directed. In two experiments the P approximated the R waves, and in one the P waves disappeared. An example of the marked changes which occurred in the electrocardiograms of this group of cases is given in figures 22 to 24.

DISCUSSION.

The experiments which have been described show that in the anaphylactic reaction produced by intravenous injections of horse serum into sensitized rabbits, disturbances of the heart occur in a

large majority of cases. These disturbances are seen in non-fatal as well as in fatal anaphylaxis, and after the vagi are cut as well as when they are intact. There were but two negative experiments in twenty-four, and in these the rabbits showed no signs of anaphylaxis. It is thus evident that cardiac disturbances are practically constantly present in rabbits during the anaphylactic reaction, in contrast to dogs, where changes in the heart-beat were noted in but six of twelve experiments,[14] although a marked fall of blood pressure was constant. An intravenous injection of horse serum does not cause such cardiac disturbances in non-sensitized rabbits, nor in rabbits in the anti-anaphylactic state.

The cardiac disturbances vary considerably. They may consist of disturbances in conduction, giving rise to partial heart-block, which occasionally goes on to complete dissociation of auricles and ventricles. Partial heart-block occurred in our series only in experiments in which there was fatal anaphylaxis and in which the vagi were intact. The number of experiments is too small, however, to indicate whether or not it is only under such conditions that partial heart-block may develop. That the relationship between auricular and ventricular activities may become disturbed is indicated by the abnormal proximity of the P and R waves (figure 15). This change, which was seen only once in the series on dogs, occurred in seven experiments of this series. Its significance has been discussed in our paper on cardiac anaphylaxis in the dog; it probably represents a change in the position of the point at which the stimulus of the heart-beat arises.

The changes in form of the electrocardiograms, especially of the portions representing ventricular systole, are various. The waves most frequently altered are the T waves, which disappeared in six experiments, became negative or downwardly directed in five, and increased in size in four. In eleven experiments, negative or downwardly directed S waves developed during the anaphylactic reaction. The changes in the form of the electrocardiograms suggest damage to the ventricles, which renders their contractions abnormal. A discussion of the various forms encountered is not in the scope of this paper. It may be said, however, that changes occur similar

[14] Robinson, G. C., and Auer, J., *loc. cit.*

to those obtained by Eppinger and Rothberger[15] following the injections of toxic substances directly into the ventricular musculature of the dog.

The cardiac disturbances in sensitized rabbits occur very soon after the intravenous injections of serum, in some experiments before the end of the injection. They were usually observed from thirty seconds to three minutes after the beginning of the injection, although in a few experiments five minutes elapsed before cardiac disturbances occurred.

The heart apparently recovers quickly in the non-fatal experiments, and electrocardiograms similar to those obtained before the injection appeared from two and a half to eighteen minutes after the abnormalities set in. The oscillation between normal and abnormal forms of the electrocardiograms was usually gradual, but in several experiments marked abnormalities occurred or disappeared suddenly. In the fatal cases it was also noted that early occurring abnormalities tended to disappear before the changes of the dying heart set in. This was seen especially well in those experiments where partial heart-block occurred, for the normal sequential beat was usually reëstablished once or even twice before the final changes of cardiac death appeared.

SUMMARY.

Electrocardiographic examination of rabbits during the anaphylactic reaction revealed marked and various changes of the heart's activity in twenty-two out of twenty-four animals. Changes occurred in fatal as well as in non-fatal cases, after the vagi were cut as well as when they were intact. Cardiac disturbances are thus a practically constant result of serum anaphylaxis in the rabbit. It therefore is possible that anaphylaxis plays a rôle in the causation of certain cardiac derangements in man.

[15] Eppinger, H., and Rothberger, C. J., *Wien. klin. Wchnschr.*, 1909, xxii, 1091.

EXPLANATION OF PLATES.[16]

PLATE 33.

FIG. 1. Experiment 8. Electrocardiogram taken 1 minute after the beginning of the injection. Rate = 233. P-R time = 0.075 of a second.

FIG. 2. Experiment 8. ½ minute later, and before the end of the injection. Auricular rate = 225. Ventricular rate = 112. Partial heart-block.

FIG. 3. Experiment 8. 2¾ minutes after the beginning of the injection. Rate = 300. P-R time = 0.06 of a second. Partial heart-block has disappeared. T wave has diminished. S wave has developed.

FIG. 4. Experiment 8. 4 minutes after the beginning of the injection. Auricular rate = 300. Ventricular rate = 150. Partial heart-block has returned. P waves indefinite. Well-marked negative or downwardly directed T waves.

FIG. 5. Experiment 8. 5 minutes after the beginning of the injection. Rate = 205. Partial block has again disappeared. P-R time = 0.072 to 0.08 of a second. Respiration ceased half a minute later.

FIG. 6. Experiment 8. 5¾ minutes after the beginning of the injection. Auricular rate = 312. Partial heart-block developing.

FIG. 7. Experiment 8. ½ minute later. Partial heart-block. Auricular rate = 300. Ventricular rate = 150.

[16] Time is marked in 1/5 and 1/50 of a second.

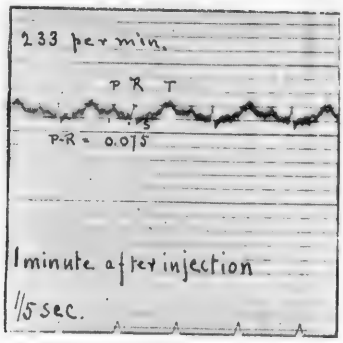
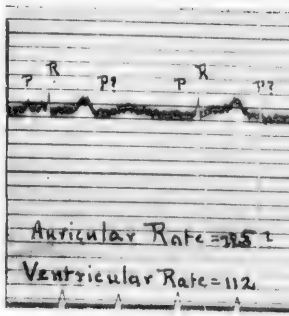


FIG. 1.



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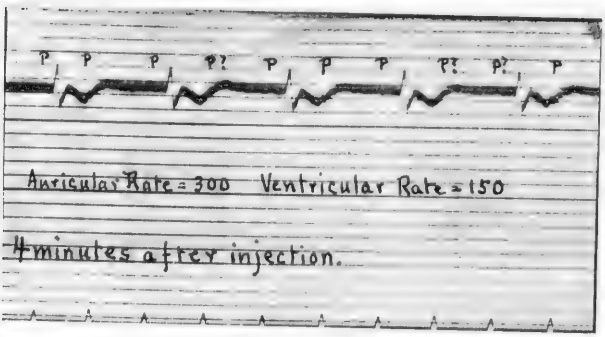
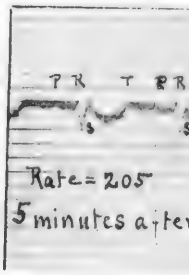


FIG. 4.



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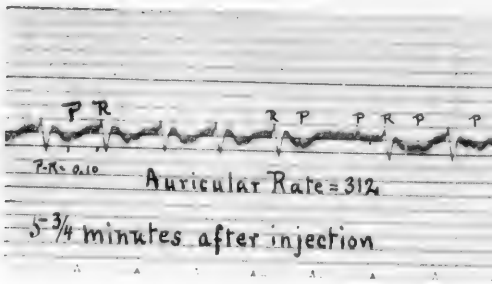


FIG

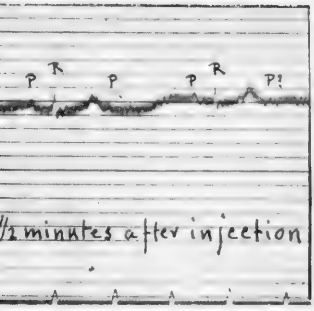


FIG. 2.

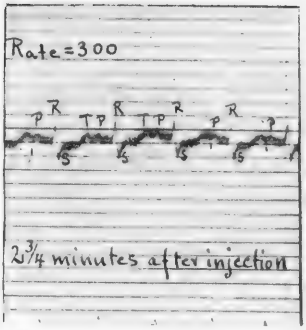


FIG. 3.

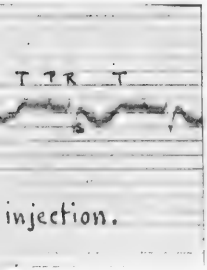


FIG. 5.

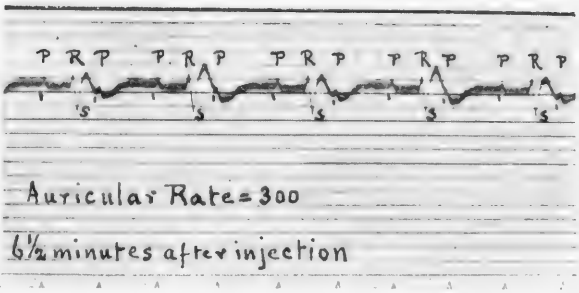


FIG. 7.

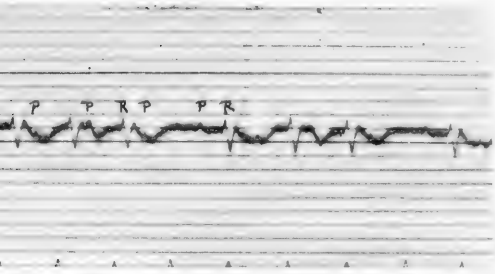


FIG. 6.

(Auer and Robinson: Electrocardiographic Study of the Anaphylactic Rabbit.)

PLATE 34.

FIG. 8. Experiment 3. Electrocardiogram taken before injection. Rate = 300. P-R time = 0.066 of a second.

FIG. 9. Experiment 3. 1½ minutes after the beginning of the injection. Auricular rate = 280. Ventricular rate = 140. Partial heart-block. The ventricular complexes have the form often caused by dying hearts.

FIG. 10. Experiment 3. 3 minutes after the beginning of the injection. Auricular rate = 105. Ventricular rate = 108. Complete dissociation of auricles and ventricles.

FIG. 11. Experiment 3. 4 minutes after the beginning of the injection. Rate = 261. Heart-block has disappeared. P-R time = 0.11 of a second.

FIG. 12. Experiment 3. 4½ minutes after the beginning of the injection. Auricular rate = 255. Partial heart-block. 2 : 1 rhythm changing over to 3 : 1 rhythm.

FIG. 13. Experiment 3. 11 minutes after the beginning of the injection. 1 minute after respiration had ceased. The form of the ventricular portions of the electrocardiograms resembles those seen in figure 9. Partial heart-block.

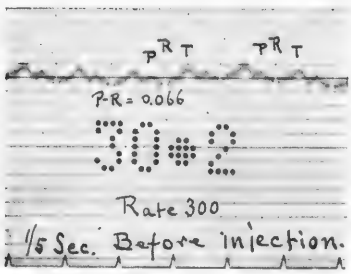


FIG. 8.

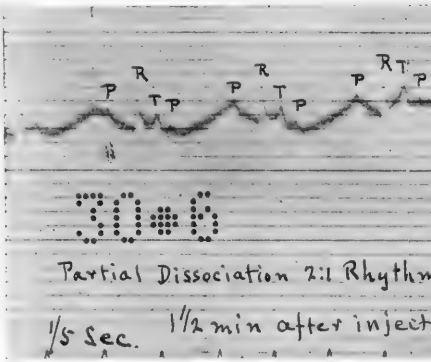


FIG. 9.

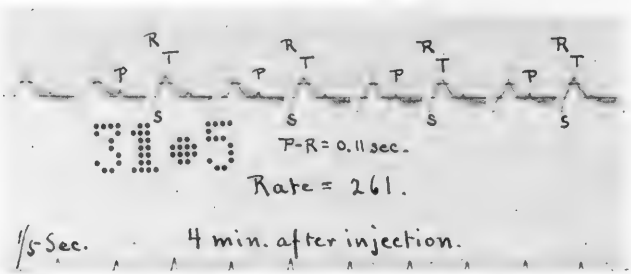
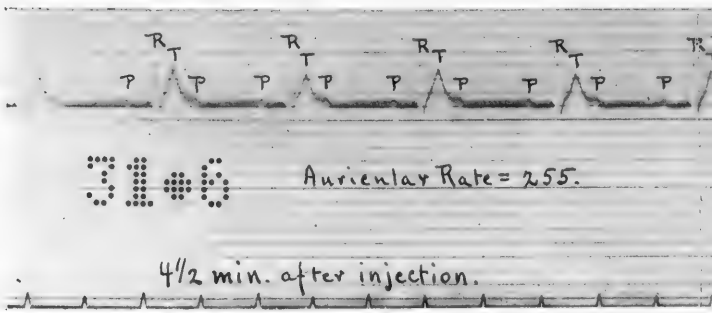


FIG. 11.



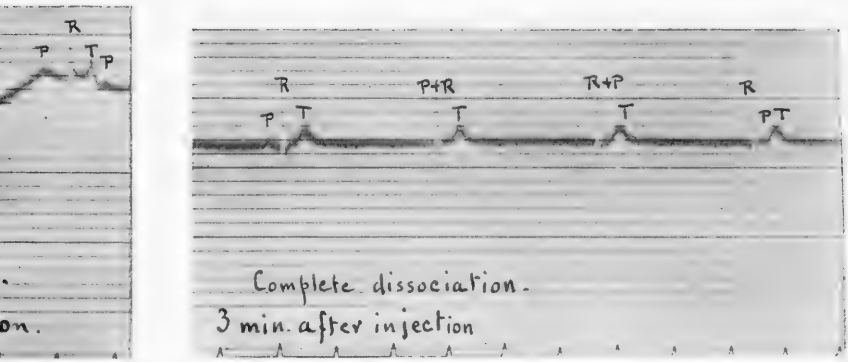


FIG. 10.

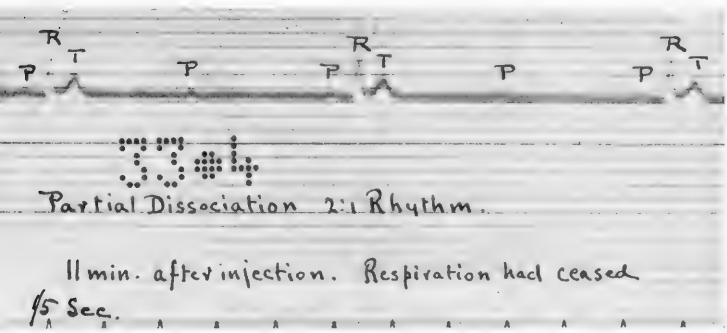
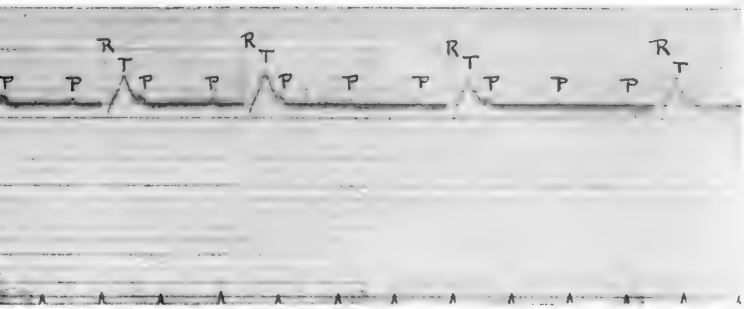


FIG. 13.



G. 12.

(Auer and Robinson: Electrocardiographic Study of the Anaphylactic Rabbit.)

PLATE 35.

FIG. 14. Experiment 6. Electrocardiogram obtained before injection. Rate = 214. P-R time = 0.08 of a second.

FIG. 15. Experiment 6. 2 minutes after the beginning of the injection. Rate = 212. P-R time = 0.033 of a second. Abnormal relation of auricular and ventricular activity.

FIG. 16. Experiment 10. Before injection. Rate = 270. P-R time = 0.064 of a second. T wave well formed.

FIG. 17. Experiment 10. 5 minutes after the beginning of the injection. Rate = 265. P-R time = 0.055 of a second. T wave has become negative or downwardly directed.

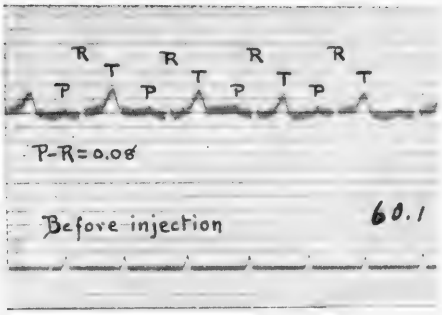


FIG. 14.

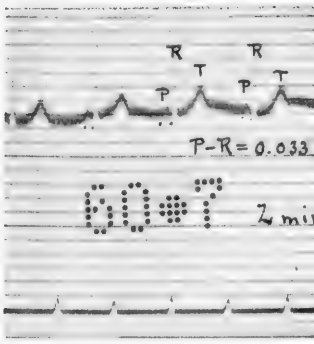


FIG. 15.

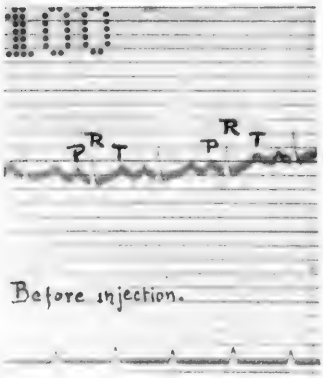


FIG. 16.

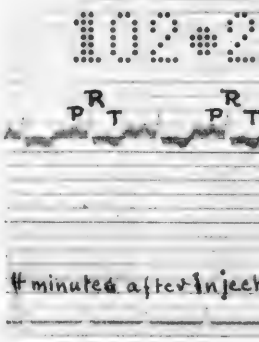
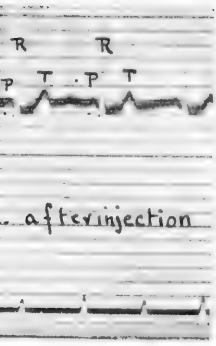
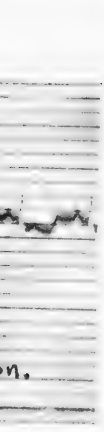


FIG. 17.

(Auer and Robinson: Electrocardiographic Study

PLATE 35.





f the Anaphylactic Rabbit.)

PLATE 36.

FIG. 18. Experiment 11. Before injection. Rate = 281. P-R time = 0.07 of a second.

FIG. 19. Experiment 11. 3¼ minutes after the beginning of the injection. Rate = 174. P-R time = 0.07 of a second. The form of the ventricular portions of the electrocardiograms is changed, the R wave becoming larger and being followed by a broad depression.

FIG. 20. Experiment 11. 5½ minutes after the beginning of the injection. Rate = 279. P-R time = 0.05 of a second. Ventricular portions of electrocardiograms markedly abnormal.

FIG. 21. Experiment 11. 20 minutes after the beginning of the injection. Rate = 260. P-R time = 0.07 of a second. The electrocardiograms resemble those of the control curve. Rabbit recovered.

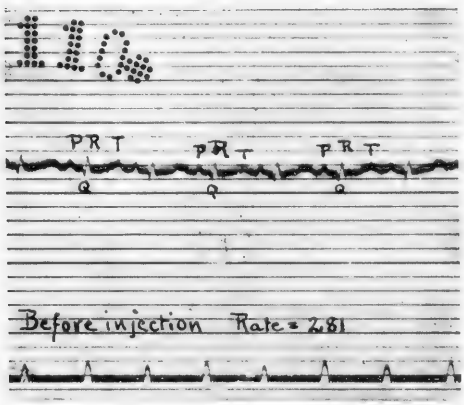


FIG. 18.

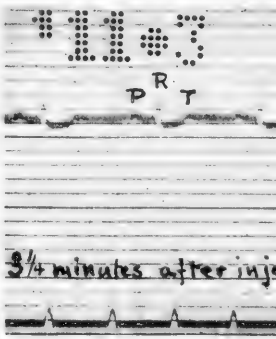


FIG. 19

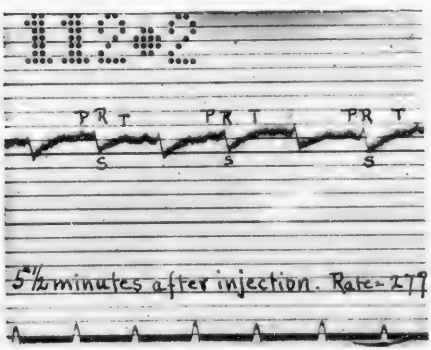


FIG. 20.

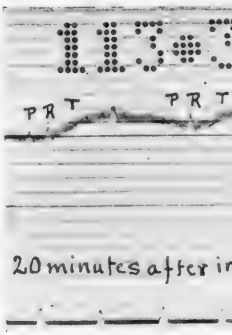
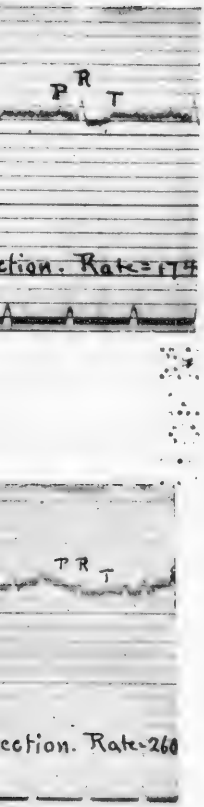


FIG.

(Auer and Robinson: Electrocardiographic Study

PLATE 36.



the Anaphylactic Rabbit.)

PLATE 37.

FIG. 22. Experiment 24. Before injection. Rate = 271. P-R time = 0.06 of a second. The electrocardiogram is normal.

FIG. 23. Experiment 24. 2 minutes after the beginning of the injection. Rate = 281. P-R time = 0.04 of a second. Ventricular portions of the electrocardiograms become suddenly very abnormal.

FIG. 24. Experiment 24. 5 minutes after the beginning of the injection. Rate = 262. P-R time = 0.06 of a second. The electrocardiograms return suddenly to the form obtained before the injection.

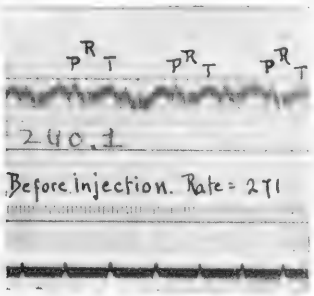


FIG. 22.

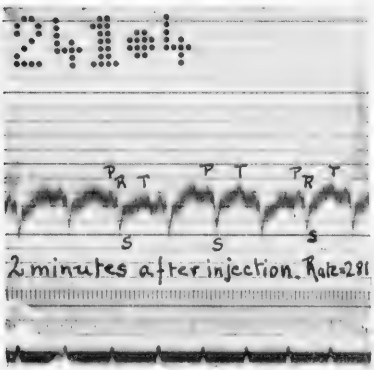


FIG. 23.

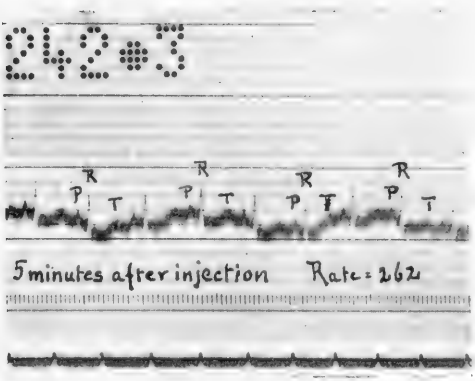


FIG. 24.

(Auer and Robinson: Electrocardiographic Study of the Anaphylactic Rabbit.

EXPERIMENTS ON THE CULTIVATION OF THE MICROÖRGANISM CAUSING EPIDEMIC POLIOMYELITIS.*

By SIMON FLEXNER, M.D., and HIDEYO NOGUCHI, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

Plates 38 to 41.

Several attempts have been made to demonstrate under the microscope and to develop in artificial cultures the microörganism causing epidemic poliomyelitis, but hitherto unsuccessfully. Giersvold[1] cultivated certain micrococci from the cerebrospinal fluid, and Fox[2] a bacillus from the circulating blood of poliomyelitic patients, but both have now been discredited as causes of the disease. The discovery of the filterable nature of the causative agent independently by Flexner and Lewis[3] and Landsteiner and Levaditi[4] not only disposed finally of the claims made for the above mentioned bacteria, but also discouraged renewed efforts at cultivation. Flexner and Lewis[5] noted and Levaditi[6] confirmed a clouding of serum bouillon by an aqueous Berkefeld filtrate of the central nervous tissues of poliomyelitic monkeys, but the phenomenon proved to be due to protein precipitation and not to multiplication of a living parasite. Proescher[7] has recently stained certain coccus-like bodies in films prepared from the central nervous organs of monkeys infected experimentally with the virus of poliomyelitis. The precise nature of these bodies has still to be worked out; ap-

* Received for publication, June 20, 1913.

1 Giersvold, M., *Norsk Mag. f. Lægevidensk.*, 1905, iii, 1280.

2 Dixon, S. G., Fox, H., and Rucker, J. B., *Pennsylvania Department of Health, Laboratory Report,* March 2, 1911.

3 Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 2095.

4 Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. de biol.*, 1909, lxvii, 592.

5 Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1910, liv, 45.

6 Levaditi, C., *Presse méd.*, 1910, xviii, 44.

7 Proescher, F., *New York Med. Jour.*, 1913, xcvii, 741.

parently they have not yet been demonstrated in human nervous tissues, and similar bodies have been found in the nervous organs of animals experimentally infected with rabic materials. The comparatively large size of many of these coccoid bodies leaves it doubtful whether they can bear relation to filterable viruses.

The successful cultivation of spirochætæ, both parasitic and saprophytic, by Noguchi[8] provided the impulse for a renewed attempt at the cultivation of the microörganismal cause of poliomyelitis. The application by Noguchi of his method to the central nervous tissues derived both from human and from experimental (monkey) examples of poliomyelitis has yielded results of an entirely different order from those previously obtained. The results have already been described briefly in our Fifteenth Note[9] and they will be described more fully in this place.

NATURE AND SOURCES OF MATERIALS.

During the several years that epidemic poliomyelitis has prevailed in the eastern United States we have collected many specimens from affected human beings and monkeys. Portions of nervous and other organs were regularly set aside in 50 per cent. sterile glycerin in the refrigerator. Hence there were available for the cultivation tests both fresh specimens yielded by recent autopsies upon human beings and monkeys and material preserved for periods ranging from a few days to many months. The new experiments upon cultivation were begun during the summer of 1912, at which time many acute cases of poliomyelitis were being cared for in the Hospital of the Rockefeller Institute, where a number of autopsies came to be performed upon fatal cases. Thus the cultivation was carried out simultaneously upon fresh, sterile, or practically sterile (for ordinary bacteria) specimens derived from human beings and from monkeys experimentally inoculated, and upon glycerinated specimens from both sources.

The material subjected to cultivation should be taken as soon after the death of the person or animal as possible, and should be obtained when possible in an aseptic condition. While any part

[8] Noguchi, H., *Jour. Exper. Med.*, 1911, xiv, 99; 1912, xv, 90; xvi, 199, 211.
[9] Flexner, S., and Noguchi, H., *Jour. Am. Med. Assn.*, 1913, lx, 362.

of the central nervous system is suitable, the brain is to be preferred for the reason that it can be obtained more readily than the spinal cord or the intervertebral ganglia in an aseptic state. The dura covering one of the hemispheres having been seared with a hot instrument is incised with sterile instruments and the underlying cortex scrupulously exposed. From this a piece of about two cubic centimeters extending into the white matter is excised and placed at once into a sterile dish. If desired, portions of the cerebellum may be obtained in a similar manner, and by removing the cerebral hemispheres and then lifting up the cerebellum the medulla may be exposed in an aseptic manner, so that portions may also be taken.

It is advisable to make the initial inoculation with fragments as well as with emulsions or with filtrates of the nervous tissues. The fragments are to be preferred since they require almost no manipulation. When, however, the original material is suspected or known to be contaminated, it is advisable to employ filtrates at the same time. The filtrates are prepared by grinding the nervous tissue in strength of about 5 per cent. with sand in distilled water or physiological salt solution. The resulting emulsion is shaken in the machine for about thirty minutes, after which the suspended matter is removed by centrifugalization and the supernatant fluid is pressed through a Berkefeld filter, N. or V.

METHOD OF CULTIVATION.

The culture medium consists essentially of human ascitic fluid, to which has been added a fragment of sterile fresh tissue. In contradistinction to the spirochætæ, for which sheep serum water supplies a suitable medium, the microörganism isolated from the nervous organs of cases of poliomyelitis demands ascitic fluid. In obtaining the initial culture, the exclusion of oxygen is necessary, but it is not, as in the case of *Treponema pallidum,* essential that the inoculated tubes should be placed in an anaerobic jar; it suffices to cover the liquid with a deep layer of sterile paraffin oil. In some instances, however, the initial growth can be obtained more certainly when the anaerobic jar is employed.

Test-tubes measuring 1.5 by 20 centimeters are chosen. Into

each of a series a fragment of the usual size of sterile fresh kidney of the normal rabbit is placed. Next is added a fragment of corresponding size of the cerebrum or other part of the brain or spinal cord. Upon these are poured about fifteen cubic centimeters of sterile ascitic fluid and finally about four cubic centimeters of the sterile paraffin oil. The ascitic fluid must have been originally sterile, since sterilization by fractional heating or filtration renders it unsuitable. The control tubes consist of the ascitic fluid and kidney fragment without brain tissue and ascitic fluid plus brain tissue without the kidney fragment. It is best to prepare two sets of the cultures, one to be placed in the anaerobic jar and the other kept outside. The incubation is conducted at the temperature of the thermostat, namely, at 37° C.

The culture tubes in the anaerobic jars should not be disturbed for from seven to twelve days, while those not within the jar may be inspected daily. Any tube showing marked turbidity, coagulation, or gas production within one or two days may be set aside as grossly contaminated. The tubes which remain clear or show only slight turbidity may none the less be contaminated. To exclude contamination small quantities of the medium are removed with pipettes and stained for bacteria in the ordinary way and cultivated upon the usual solid or fluid media. Obvious bacteria in the films as well as any growth taking place in the ordinary media in the presence of air may be put down as contaminations and the tubes containing them discarded.

Gross contaminations having been excluded, the peculiar microorganism obtained from the poliomyelitic nervous tissues exhibits the following growth characters. At the expiration of about five days' incubation a faint opalescence appears about the fragments of tissue at the bottom of the tube. This opalescence can be gradually diffused through the tube by gentle shaking (agitation), in the course of which it is observed that the turbidity about the tissues was really greater than was at first apparent (figure 1, fluid culture). The control tubes when not grossly contaminated either remain perfectly clear or a slight granular precipitate of washed out particles of tissue forms about them. The two appearances are quite distinct and become more pronounced after another period of

three to five days, during which time the opalescence first described extends into the upper portion of the medium, while in the controls the precipitate gathers more and more at the lower end of the tubes. At the expiration of ten or twelve days the diffuse opalescence of the medium in the tubes in which growth has been observed to take place begins to diminish as sedimentation sets in, during which minute, irregular particles form, heavy enough to fall slowly to the bottom of the tube.

The corresponding tubes contained in the anaerobic jar when examined on the seventh day show a similar opalescent growth but slighter than that just described. When replaced in the jar and returned to the thermostat the opalescence increases so that at the expiration of another period of five or six days it approximates that present at the end of one week in the tubes kept outside the jar.

Cultivation may also be performed in a solid medium, consisting of ascitic fluid and sterile rabbit tissue, to which a suitable quantity of 2 per cent. nutrient agar has been added in order to produce a solid mixture. The melted agar, cooled to a temperature of 40° C., is mixed with the ascitic fluid in the tube containing the fragment of tissue, also previously heated to 40° C. After cooling and solidification the medium should be sufficiently firm to maintain its stability. In this medium an initial growth, aside from contaminations, from poliomyelitic material has never been obtained, but once growth has been secured in the fluid medium it is possible, but not in all instances, to transmit it to the solid medium. Often successful transfer from the fluid to the solid medium is not accomplished until about the third generation of the growth has been obtained in the fluid medium. The changes indicative of growth in the solid medium begin about the fragment of tissue and appear first as a diffuse opalescence which gradually aggregates into visible grains of minute size that are recognized as colonies. Several days are usually required before this initial change in the medium becomes apparent. The rapidity with which it does become apparent is affected by the generation of the culture and hence by its adaptation to the medium. Gradually the opalescence rises in the medium within the tube, until it reaches within about three centimeters of the surface of the medium, at which a sharp line of demarcation

appears. When the number of colonies developing is large the individuals are so minute as to give a diffuse grayish appearance to the medium, which requires a lens to bring out the separate colonies. When the number of colonies developing is smaller they may reach a diameter of about one third of a millimeter and hence become visible as such to the naked eye, to which they appear round in shape and grayish white in color. Under favorable conditions the opalescence indicating growth about the tissue fragment becomes apparent at the end of forty-eight hours and distinct after seventy-two to ninety-six hours, although several days may be required before the ascent of the growth to the line of demarcation, at which the presence of oxygen brings about a cessation of development (figure 2, solid culture).

The description just given relates to cultivation with fragments of fresh central nervous organs in the ascitic fluid and in the presence of sterile rabbit tissue. It is, however, possible to secure cultivation even in the absence of the rabbit tissue. For this purpose a somewhat larger fragment of the poliomyelitic brain is placed in the tubes containing the ascitic fluid, which is then covered with the layer of paraffin oil. After incubation at 37° C. the initial growth takes place, but with less constancy than when the rabbit tissue is also present. When it occurs the growth is usually so slight that the opalescence remains absent or nearly so, and it becomes necessary to transplant into a medium containing fresh rabbit tissue to establish its existence.

Just as it is possible to eliminate the fresh rabbit tissue so is it possible to dispense with the ascitic fluid, which may be substituted by an extract prepared from the brain tissue or even sheep serum water. The extract is prepared by grinding in a porcelain mill and then afterwards shaking for half an hour in a machine twenty grams of fresh normal brain of a *Macacus rhesus* monkey in 500 cubic centimeters of distilled water or Ringer solution. The emulsion is centrifugalized and the supernatant fluid passed through a Berkefeld filter, V. The clear sterile filtrate is used to replace the ascitic fluid, but in order to obtain growth in this fluid or in serum water it is necessary to employ a fragment of rabbit tissue. A successful result has never been obtained with either, in the absence of the

rabbit tissue, from which it follows that they are inferior to the ascitic fluid as a medium of cultivation.

In several parallel experiments an emulsion of the brain tissue was compared with the brain fragment as the source of the culture and it was found that growth was obtained with greater difficulty and somewhat less frequency from the emulsion than from the whole tissue fragment. While growth was detected during the first week in the corresponding tubes of fragment and emulsion, a week later the growth may have disappeared from the emulsion tubes, from which it was concluded that certain constituents of the nervous tissue, liberated by the emulsion, exerted an injurious effect upon the developing microörganism. In support of this supposition it was found that when an emulsion or extract of the brain was added to an active culture prepared in the usual way the microorganisms previously readily demonstrable in stained preparations became indistinguishable. That they do not entirely disappear under these circumstances is shown by the fact that when transplanted into the ascitic fluid tissue medium a growth reappears.

The Berkefeld filtrates of emulsified tissue also give rise to cultures, but less constantly than the original nervous material, from which the filtrates were prepared. In cultivating with the filtered extract, the ascitic fluid rabbit tissue medium should always be employed, and two sets of tubes prepared, one to be placed in the anaerobic jar and the other to be kept outside. The initial appearance of growth is somewhat delayed, so that an average of ten days of incubation may be necessary before the characteristic alteration of the medium becomes apparent. In no instance was success attained with the filtrate in the absence of the rabbit tissue.

Glycerinated human and monkey poliomyelitic tissues were employed as fragments, and, in the case of monkeys, as filtrates for cultivation. The specimens had been kept in 50 per cent. glycerin at a temperature of 2 to 4° C. for periods varying from twenty-five days to one year. Portions of the brain or spinal cord so preserved were cut off with sterile scissors, washed thoroughly in sterile distilled water, and placed in the ascitic fluid tissue medium. The resulting growths, when they occurred, produced the characteristic appearances already noted. On the whole, the glycer-

inated tissues gave somewhat more inconstant results than the fresh material.

Once the initial growth from any of the sources mentioned has been secured it is, as a rule, possible to obtain subcultures by transplanting about 0.2 of a cubic centimeter of the original culture per tube into the next series. Since the initial growth begins about the tissue at the bottom of the tube it is advisable to take the fluid for transplantation from this location. Subcultures are obtained more constantly in the fluid than in the solid medium. Hence growth will sometimes appear in the former and not in the latter. Once the microörganism has been adapted to the conditions of artificial cultivation, transplantation to the solid medium may be effected. The adaptation is indicated by a more rapid and abundant growth in the fluid medium, and its occurrence is promoted by the presence of fresh sterile tissue and is prejudiced by its absence. Among the monkey tissues was one specimen which had been glycerinated for nine months, and from which a growth was obtained with a Berkefeld filtrate.

In the course of the growth the sterile kidney turns first pink and then gray in color, but no obvious solution or hemolysis of the red corpuscles contained in the tissue occurs.

The addition of various carbohydrates and alcohols, such as glucose, saccharose, lactose, maltose, gelactose, levulose, mannite, glycogen, dextrin, inulin, dulcite, and arabinose, to the ascitic fluid or sheep serum water in proportion of 0.5 per cent. did not affect the cultivation, and the growths when pure had no apparent effect upon them, since neither acid nor gas are produced.

Litmus milk, to which fragments of the sterile fresh tissue have been added, affords a medium in which growth will take place without producing any alteration in the appearance of the medium.

NUMERICAL RESULTS OF CULTIVATION.

In a series of thirty-three experiments with the nervous tissue of monkeys, comprising thirteen specimens of brain tissue, two of brain emulsion, and eighteen filtrates of brain and spinal cord from twenty-four animals infected from two different strains of human poliomyelitic virus, an initial growth was obtained in nineteen in-

stances, of which sixteen proved to be pure and three mixed cultures. Of these, pure subcultures were obtained thirteen times, namely, eight times from brain tissues, four times from filtrates, and once only from emulsion. In a second series in which human nervous tissues were directly employed, eight successful cultivations were secured. One was from fresh tissue, three were from recently glycerinated tissues, and four from tissues which had been glycerinated for many months. In two specimens of the last four, initial cultures but no subcultures were obtained. In all the others subcultures resulted and were maintained alive for an indefinite period.

MORPHOLOGICAL CHARACTERS.

Uncontaminated cultures of the microörganism from the various human and monkey sources already mentioned possess common morphological and staining characters. Fluid cultures viewed under the dark-field microscope exhibit among the innumerable dancing protein and other granules present minute bodies, globular in form, hanging together in short chains, pairs, and small masses, devoid of independent motility and distinguishable with difficulty as a special class among the indefinite granules present. Stained preparations, on the other hand, bring out unmistakable microorganisms grouped in the three ways stated and of very minute size. Staining may be accomplished with more or less difficulty in several ways, but the two methods which have thus far yielded the most satisfactory results are those of Giemsa and Gram.

The Giemsa stain may be applied in two ways according as a rapid or a somewhat slower but better result is desired. In the first, the film, either upon a slide or a cover-glass, is allowed to become air-dry, when it is fixed over the flame, and several drops of Giemsa solution are dropped upon it and diluted with about twenty drops of distilled water. After gentle mixing of the dye and water the preparation is held over the flame until a trace of vapor rises. Care is exercised to avoid boiling. The preparation is then washed in a stream of distilled water, pressed between blotters, and dried. This method does not yield uniform staining or constancy of shade, but is useful for rapid examinations. The second method is the one usually employed. The film having been air-dried is

fixed in methyl alcohol for about an hour, washed in distilled water, and is then immersed in the solution containing one drop of the stain per cubic centimeter of distilled water, in which the film remains for from two hours to twelve to fourteen hours (over night). Gram's stain is applied in the usual way, using gentian violet as the dye and safranin as the counterstain. The microörganism behaves variably towards the Gram stain. When the medium in which it is grown contains peptone, it retains the stain more assiduously than when it is devoid of that substance. Then the intensity with which the Gram stain is retained varies with the age of the culture. The older the culture the more strongly the gentian violet is held. While the grouping is of course identical whichever stain is employed, the apparent size of the microörganism is affected by the dye. When the gentian violet is retained the individuals appear larger than they do with either the safranin or the Giemsa stain. Loeffler's alkaline methylene-blue, carbol-fuchsin, and carbol-gentian violet all stain the microörganism slightly or without affording sharp differentiation. The microscopical appearances of the microörganism vary somewhat according to whether the growth is in a fluid or solid medium and peptone is present or absent. Peptone and possibly other constituents of the bouillon render the microörganisms somewhat larger as well as enable them to retain more firmly the Gram stain.

Films prepared from the lower layer of the ascitic fluid culture about the sterile rabbit tissue and stained by Giemsa solution reveal the presence of a variable number of minute globoid bodies, arranged in pairs or short chains, or in small aggregated masses. In the fluid culture the pairs and chains predominate (figures 3, 4, and 5). According as the staining operation was carried out by the rapid or slower method, the globoid bodies assume a bluish or a violet color; rarely they present a red tint. Since the most uniform results are obtained by the slower method of staining, it may be considered that the violet coloration is the normal. The individual microörganisms average about 0.2 of a micron in diameter, the limits of visible bodies being 0.15 to 0.3 of a micron. Possibly still smaller individuals exist that exceed the limit of visibility. For purposes of comparison a group of chain forms has been compared by photography with *Streptococcus pyogenes* (figure 6).

Considerable difficulties may be encountered in demonstrating the microörganism in initial cultures not older than four or five days. Either nothing whatever that can be recognized as microörganisms may be detected, or merely roundish bodies the nature of which it may be difficult to decide. When the same tubes are examined on the sixth or seventh day the unmistakable minute globoid bodies are usually detected. Although the original growth tends to appear slowly, the final multiplication takes place rapidly, and is completed from about the eighth to the twelfth day, after which the cultures remain stationary for a variable period. Kept in the thermostat the microörganisms may remain largely unchanged for many days, and in the refrigerator for many weeks.

Within the solid cultures the microörganism develops not as chains but as pairs and aggregated groups. The arrangement is very readily ascertained by compressing a fragment of the agar between two slides, which are afterwards separated. The resulting films having been stained in the usual manner by Giemsa or Gram solution, the minute particles of agar adhering to the glass will frequently exhibit colonies, of which the members are sufficiently separated to expose the grouping (figure 7). In the Gram-stained films the bodies are somewhat coarser than in the Giemsa-stained ones. In young agar cultures the Gram-stained bodies are partly purple and partly pink, while in older cultures almost all appear purple.

After a somewhat variable period, and generally from ten days to three or four weeks after the maximum growth has been obtained, certain metamorphoses or degenerations set in. Probably the enlarged and irregular stained bodies are to be viewed as degenerations, while the minute fragments just at the limit of visibility may possibly represent a metamorphosis into a minuter variety with which submicroscopic forms may be associated. When the conditions of culture are unfavorable, as, for example, when the brain extract or other medium without sterile tissue is employed, this supposed metamorphosis appears to arise earlier, and indeed within the first few days of growth. Subcultivation from one of the altered cultures or from their Berkefeld filtrates yields frequently but not uniformly new developments of the visible microörganisms.

The important question, as to whether the cultures just mentioned contain actual ultramicroscopic forms of the microörganism, or whether the failure to detect the visible forms is due chiefly to dilution, must remain for the present undecided. What is certain, however, is that the globoid organisms are capable of passing though Berkefeld filters which exclude the usual test bacteria (*e. g., Micrococcus prodigiosus*). Cultures from the Berkefeld filtrates should always be made in the ascitic fluid tissue medium, as in the case of the initial growth.

The film preparations stained by the Giemsa stain and especially by the rapid method sometimes show the globoid organisms surrounded by a zone either faintly tinted or colorless, that may equal in width the diameter of the individual microörganisms. Single bodies, aggregated groups, or chains may be thus enveloped. The nature of the material composing the zone is unknown, as is also whether it represents an independent part of the organism (capsule?) or is derived from the culture medium.

INFECTION OF MONKEYS.

The microörganism described is present so constantly in the central nervous tissues of affected human beings and monkeys that a strong presumption exists to the effect that it bears some relationship to epidemic poliomyelitis. It is sufficiently obvious that whatever they may ultimately prove to be, as far as we now know the globoid bodies are not common pathogenic or even saprophytic microörganisms. Whether the bodies are limited to the central nervous organs or occur more widely distributed in the body of infected human beings and monkeys is still to be determined. The question which presses at the moment is whether the cultures are capable of setting up in monkeys clinical and pathological states corresponding with those characteristic of experimental poliomyelitis. Before proceeding to describe the inoculation experiments, it may be well to point out that apparently the globoid bodies are highly parasitic microörganisms. In view of this circumstance it may readily happen that they may quickly lose pathogenic properties in artificial cultures. There are of course several instances known among the bacteria and spirochætæ of which this circum-

stance is true. We were therefore prepared to encounter difficulties in the way of infecting monkeys with cultures. It is necessary to recall the difficulties surrounding the original implantation of highly infected human poliomyelitic material upon monkeys in order to have presented emphatically the probable obstacles that stand in the way of the successful accomplishment of the infection of monkeys with the cultures.

Two series of inoculations were made. In one the cultures derived from the nervous tissues of monkeys and in the other from human beings directly were separately inoculated into *Macacus rhesus* monkeys. The inoculations were made either into the brain or into the sciatic nerve and peritoneal cavity simultaneously.

Before describing the several instances of successful inoculation, certain important points should be presented. It is known that the virus in an emulsion or filtrate prepared from the nervous tissues of infected monkeys survives in the thermostat and remains infectious for at least thirty days, and, also, that the filtrate soon becomes inactive after being transferred to a new fluid medium consisting of serum bouillon. Possibly the inactivity arises chiefly from the great dilution which the virus has undergone. Whence it follows that dilutions of the virus beyond the point at which infection through intracerebral inoculation of several cubic centimeters of suspension can be produced are easily secured. We have frequently had occasion to confirm this general fact, since second transfers into the fluid medium from a supposed culture derived from monkeys previously inoculated with the highly active M A virus have in many instances failed to cause infection even when injected intracerebrally. Dilution beyond the point of infectivity of the virus, even in the culture media, thus is readily accomplished.

Bearing these facts in mind it follows that successful inoculation with culture generations still more remote than the second dilution of the original virus is to be interpreted as representing, probably, an implantation of the artificially cultivated microörganism upon the susceptible monkeys. Moreover, from the monkeys in which experimental poliomyelitis has been thus produced the microörganism introduced can be recovered in culture and even implanted successfully again.

The implantation upon monkeys of the virus contained in the original human nervous tissues is admittedly difficult. When filtrates are inoculated failure usually follows. When emulsions are employed, infection occurs in from about 50 to 100 per cent. of the instances, probably depending upon variation in the degree of infectivity of the original virus. The human strains of the virus are less infectious for monkeys than the adapted or so called monkey strains, a fact that is the more significant as respects the successful inoculation of cultures derived directly from human tissues.

Cultivated Human Strains.—Fluid and solid cultures in the third generation from four human sources, namely, D., Kr., Ko., and F., were mixed. Each culture had been obtained from recently glycerinated specimens of the central nervous system.

Experiment A.—Macacus rhesus. December 27, 1912. 0.5 c.c. of the mixture was injected into the left sciatic nerve and 0.8 c.c. into the left cerebral hemisphere.

December 30, 1912. Animal less active than normally; legs and arms weak.

December 31, 1912. Excitable; uses legs awkwardly. Etherized.

Autopsy.—At the site of intracerebral inoculation punctiform hemorrhages; the left lateral ventricle contains a small, dark clot; the cervical, dorsal, and lumbar spinal cord appear congested; the left sciatic nerve is slightly swollen at site of injection. Film preparations from the two inoculation sites show no ordinary bacteria. The microscopical examination of the medulla, cervical, dorsal, and lumbar levels of the spinal cord, and intervertebral ganglia from the cervical and lumbar regions shows in all typical and marked characteristic lesions of experimental poliomyelitis. The lesions consist of degeneration of the nerve cells, associated with neurophagocytosis, infiltration with mononuclear cells of the ground substance, and perivascular and meningeal infiltration (figures 8, 9, and 10).

Experiment B.—Macacus rhesus. December 31, 1912. A suspension of the medulla and spinal cord of monkey A was injected into left sciatic nerve and left cerebral hemisphere.

January 1 to January 4, 1913. Increased excitability.

January 6, 1913. Excitable; vision defective; ataxia.

January 7, 1913. Arms weak, right more than left.

January 8, 1913. 9.30 A.M., prostrate; respiratory paralysis. Death at 10 A.M.

Autopsy.—A small cyst with clear contents at the site of injection. Sciatic wound healed. Congestion of gray substance of spinal cord.

The microscopical examination exhibits marked lesions of experimental poliomyelitis in the spinal cord, medulla, and intervertebral ganglia. Portions of the nervous system were placed in 50 per cent. sterile glycerin.

Experiment C.—Macacus rhesus. May 14, 1913. Inoculated intracerebrally with emulsion of glycerinated spinal cord and medulla of monkey B.

May 18, 1913. Excitable.
May 19, 1913. Arms weak.
May 20, 1913. Arms and legs paralyzed.
May 21, 1913. Etherized.

Autopsy.—A small cyst at the site of inoculation. At various levels of the spinal cord are visible lesions.

Microscopical examination shows marked and characteristic lesions of experimental poliomyelitis in the medulla, spinal cord, and intervertebral ganglia.

Cultures in the ascitic fluid tissue medium were made at autopsy from monkeys A, B, and C. From monkeys A and B the original microörganism injected was recovered in pure culture. The culture from monkey C was unsuccessful. Hence another portion of the original glycerinated material was inoculated as follows:

Experiment D.—Macacus rhesus. June 7, 1913. Intracerebral injection of emulsion of glycerinated brain and cord from monkey B.

June 12, 1913. Excitable; tremor; prostrate.
June 13, 1913. Etherized.

Autopsy.—A small cyst at the site of inoculation; visible lesions in the spinal cord.

Microscopical examination shows typical lesions of experimental poliomyelitis in the medulla, spinal cord, and intervertebral ganglia.

Cultures in the ascitic fluid tissue medium from this animal gave a pure growth of the microörganism originally injected into monkey A, the beginning of this series, more than six months previously. The successful culture in this instance indicates that the failure to recover the microörganism from monkey C was due to a technical fault.

Cultivated Monkey Strains.—These strains consisted of cultivations from the highly adapted M A virus which had been long propagated in monkeys from emulsions or filtrates of the nervous organs. Hence the cultures, while representing the same original virus, varied in precise generation according to the period at which they were isolated. All were far removed from the original human strain.

Experiment E.—Macacus rhesus. December 3, 1912. Two cultivated strains, one in the third and the other in the fifth generation, were combined, and of the mixture 1 c.c. was injected into each sciatic nerve, and 10 c.c. were injected into the peritoneal cavity.

December 6, 1912. Excitable; coat roughened.
December 9, 1912. Somewhat less excitable.

December 23, 1912. Again excitable. The monkey remained excitable continuously, and on March 19, 1913, was found in convulsions, which recurred at frequent intervals, until on March 25 death occurred.

The microscopical examination reveals focal lesions in the medulla, consisting of minute hemorrhages, small aggregations of mononuclear cells, partly in the ground substance and partly about the small vessels, and a few small vascular thrombi. Small hemorrhages also occur in the crura cerebri, and in the cervical region of the spinal cord, in which slight perivascular infiltration with mononuclear cells is also present.

Cultures yielded the original microörganism.

The interpretation of experiment D is not perfectly clear. The long intervening period between the inoculation and the development of convulsions and occurrence of death is not strictly paralleled by the effects of direct inoculation of either emulsion or filtrate of the nervous tissues. Moreover, thrombi do not occur in uncomplicated instances of experimental poliomyelitis. On the other hand the infiltrative lesions are similar to those present in experimental poliomyelitis.

Experiment F.—Macacus rhesus. December 27, 1912. Injected into left cerebral hemisphere 0.8 c.c. of cultivated virus, fifth generation.

January 1, 1913. Excitable; arms weak.

January 2, 1913. Excitability continues; arms and back partly paralyzed; tremor of head.

January 3, 1913. Legs weak.

January 4, 1913. Animal prostrate. Etherized.

Autopsy.—A small cyst at the site of injection. Spinal cord edematous. No visceral lesions.

Microscopical examination shows marked characteristic degenerative and infiltrative lesions of experimental poliomyelitis in the medulla, spinal cord, and intervertebral ganglia.

Cultures yielded the original microörganism.

Experiment G.—Macacus rhesus. January 27, 1913. Injected into left cerebral hemisphere 2.5 c.c. of culture in fifth generation from monkey E.

February 1, 1913. Excitable; movements uncertain; vision appears to be defective.

February 2, 1913. Neck and back paralyzed; unable to sit up; convulsions in course of which death occurs.

Autopsy.—February 3, 1913. The general viscera are apparently normal. A small cyst the size of an almond is present at the point of inoculation in left hemisphere. Gray matter of medulla and spinal cord is congested.

The microscopical examination of the medulla, spinal cord, and intervertebral ganglia shows typical lesions of experimental poliomyelitis.

Cultures yielded the original microörganism.

Experiment H.—Macacus cynomolgus. February 5, 1913. Injected into the left cerebral hemisphere 2.5 c.c. of a Berkefeld filtrate prepared from the site of inoculation of monkey G.

February 6, 1913. Shows tendency to incline toward right side; does not use the right arm, which is spastic, the result probably of injury from the injection.

February 7, 1913. Death at 10 A.M.

Autopsy.—At the site of inoculation there is a cyst containing blood stained fluid communicating with the lateral ventricle.

Microscopical examination shows marked lesions in the intervertebral ganglia and lesions of the blood vessels adjacent to the site of inoculation resembling those characteristic of experimental poliomyelitis. The spinal cord also reveals fine lesions of the same nature. Hence it appears that within the short period of forty-eight hours the specific lesions developed throughout a wide area of the nervous system of this animal.

Experiment I.—Macacus rhesus. February 8, 1913. Repetition of experiment G. Injected into the left cerebral hemisphere 1 c.c. of filtrate from site of inoculation of monkey F.

February 14, 1913. Prostrate; legs, back, and arms paralyzed. Etherized.

Autopsy.—A small, blood-containing cyst at the site of inoculation. Spinal cord throughout shows gross lesions of the gray matter.

The microscopical examination confirms the character of these lesions. Spinal cord, intervertebral ganglia, and medulla all show marked degenerative and infiltrative lesions of poliomyelitis, and identical lesions occur in the crura cerebri and in the cerebral hemispheres.

Experiment J.—Macacus rhesus. February 13, 1913. Suspension of glycerinated brain and spinal cord of monkey F injected intracerebrally.

February 18, 1913. Death.

Autopsy.—A cyst the size of an almond containing clotted blood occurs at the site of inoculation. Throughout the spinal cord the gray matter is congested.

Microscopical examination shows marked characteristic lesions of experimental poliomyelitis.

Experiment K.—Macacus rhesus. May 14, 1913. Injected intracerebrally with an emulsion of glycerinated spinal cord and medulla obtained from monkey E.

May 18, 1913. Distinctly weak.

May 19, 1913. Ataxic.

May 20, 1913. Paralysis of arms, legs, and back.

May 21, 1913. Etherized.

Autopsy.—At the site of inoculation there is a small cyst containing blood. Visible lesions in the spinal cord.

Microscopic examination reveals marked characteristic poliomyelitic lesions of the medulla, spinal cord, and intervertebral ganglia.

Culture yielded microörganism identical with that inoculated in monkey F almost six months previously. This culture in the fourth generation was inoculated intracerebrally on June 11, into a *Macacus rhesus.* On June 16 the monkey was weak, slow, and ataxic; it was etherized on July 2. The microscope reveals typical lesions of the brain and spinal ganglia, or, in other words, evidences of the encephalitic type of poliomyelitis.

Experiment L.—Macacus rhesus. January 13, 1913. Injected into left cerebral hemisphere 1 c.c. of cultivated virus, sixth generation.

January 16, 1913. Movements irregular; tends to move in circles.

January 17, 1913. Apparently normal.

February 6, 1913. Ataxic; excitable, arms and legs weak.

February 10, 1913. Weakness of arms and legs increased. Etherized.

Autopsy.—No lesion at the site of inoculation, and no gross changes in the spinal cord.

Microscopical examination shows fine infiltrative and degenerative lesions of the medulla, spinal cord, brain, and intervertebral ganglia.

Culture yielded microörganisms identical with that inoculated.

Experiment M.—Macacus rhesus. February 11, 1913. Injected emulsion of the spinal cord and brain of monkey K into the sciatic nerve and peritoneal cavity.

February 14, 1913. Excitable; convulsions.

February 15, 1913. Legs weak.

March 1, 1913. Excitability continues; movements slow and restricted.

March 5, 1913. Condition unchanged. Etherized.

Microscopical examination shows typical poliomyelitic lesions of the intervertebral ganglia in the lumbar and cervical regions, and similar but inconspicuous lesions of the spinal cord.

Experiment N.—Macacus rhesus. February 27, 1913. Inoculated into left hemisphere with 2 c.c. of monkey culture from two sources in the eighteenth and twentieth generation respectively.

March 1, 1913. Inclines slightly towards the right side.

March 2, 1913. Death.

Autopsy.—At the site of inoculation is a cyst the size of an almond containing clear fluid and communicating with the lateral ventricle; gray matter of the spinal cord is congested.

Microscopical examination of the cyst shows the presence of a Gram-positive coccus (mixed infection).

Experiment O.—Macacus cynomolgus. March 4, 1913. 2 c.c. of Berkefeld filtrate from the site of inoculation of monkey N are injected intracerebrally, and 100 c.c. intraperitoneally.

March 17, 1913. Left arm weak; legs somewhat spastic.

March 18, 1913. All extremities weak and spastic.

March 19, 1913. Head retracted; convulsions; unable to rise. Etherized.

Autopsy.—No lesion at the site of inoculation. A *placque jaune* 1.5 by 2 cm. occupies the surface of the cortex on the left side, posterior to the motor area. General viscera normal.

Microscopical examination reveals characteristic focal lesions of experimental poliomyelitis in the intervertebral ganglia, and vascular and interstitial lesions of the spinal cord, crura cerebri, and cerebral hemispheres.

Because of contamination with micrococci at the inoculation site in monkey N, a filtrate prepared from the local site was injected into monkey O, resulting in combined flaccid and spastic paralysis. However, the sections of the nervous tissues of monkey O by showing lesions of experimental poliomyelitis indicate the existence of that disease.

The preceding experiments show unmistakably that the inoculation of the cultures is followed by the appearance of the clinical symptoms and pathological effects characteristic of experimental poliomyelitis in the monkey. The two methods of inoculation now chiefly practised with the usual suspensions or filtrates of nervous tissues were employed successfully with the cultures. The essential results are identical whether the cultures are inoculated into the brain or simultaneously into sciatic nerve and peritoneal cavity. The inoculation of the cultures produces in monkeys no direct effect. Immediately after injection the animals appear normal and they remain apparently in this condition for an incubation period which may be as brief as forty-eight hours, but commonly endures for one to several weeks, at the expiration of which the usual symptoms of excitement, tremor, weak and then paralyzed muscles succeed one another. Death may arise from prostration, convulsions, or paralysis of the respiration, although in most of the instances related it was artificially hastened by etherization.

The central nervous tissues of the monkeys may be employed for recultivation of the microörganism inoculated, or for transmitting the experimental affection to still other and probably an indefinite series of monkeys. This transmission can be accomplished either with emulsions or Berkefeld filtrates of the fresh or glycerinated organs. Since the experimental disease is accurately reproduced in this manner, it follows that not only the cultures but the nervous tissues infected with them possess the distinctive disease-producing properties of the virus of poliomyelitis as commonly understood.

The local effects of the cultures vary with the inoculation site and the dosage. Unless secondary infection arises from the outside, the intrasciatic and intraperitoneal inoculations cause no considerable reaction, while the intracerebral injection leads to the formation of a small cyst, in which the introduced microörganism may or may not be readily discoverable in film preparations. If relatively few of the microörganisms are inoculated, they tend to disappear from view; while if many are introduced, they are readily detected, either lying free or enclosed in emigrated polynuclear or mononuclear cells (figure 11). When pyogenic micrococci enter from the skin, the local lesion tends to be larger, and to contain

many pus cells, and the two microörganisms may appear side by side, both without and within cells. When an excess of the mixed fluid and solid cultures is injected into the brain, the local effect is also more severe and many leucocytes may be called out by the positively chemotactic elements contained within the medium. The action of contaminated cultures is less readily interpreted than are the effects of pure cultures; but any doubt may be removed by preparing a Berkefeld filtrate from the local lesion and portions of the medulla, spinal cord, and intervertebral ganglia, which is to be employed for inoculation.

The disappearance of the microörganism from the inconspicuous local lesions probably results from a general diffusion throughout the nervous organs and restricted multiplication at the site of injection. The microörganism can indeed be recovered in cultures from any part of the central nervous system, and is best recovered from the intact cerebral hemisphere, where it usually exists in a pure state and is readily accessible under aseptic conditions.

RELATION OF THE MICROÖRGANISM TO NERVOUS TISSUES.

The microörganism the cultures of which have been described can be detected in film preparations and in sections prepared from the central nervous organs of human beings who have succumbed to poliomyelitis and of monkeys subjected to inoculation with the ordinary virus, or with cultures. A special technique which has been devised by Noguchi is required for the demonstration.

Staining Method for Film Impressions.—1. Cover-glasses of suitable size and thinness (No. 1 or 0) receive a succession of impressions from the selected piece of nervous tissue.

2. After becoming air-dry the cover-glass is placed, film side down, in a mixture which is freshly prepared at each operation, consisting of one part of Grübler's Giemsa solution, and two parts of Merck's methyl alcohol reagent, where it remains for two minutes. In order to prevent the film surface from contact with the bottom of the staining dish, it is well to support one end upon a drawn out glass capillary tube. The staining vessel may conveniently be a small Petri dish and cover.

3. Twenty parts of a 1 to 10,000 potassium hydrate solution are

poured into the dish at the expiration of the two minutes, and the whole is thoroughly mixed by gentle agitation. At the expiration of one hour the cover-glass is removed.

4. The film side is washed in distilled water for a few seconds, after which it is differentiated in a solution of tannic acid, consisting of one or two drops of a 20 per cent. solution, added to forty cubic centimeters of distilled water. This operation requires several seconds and careful observation.

5. The cover-glass is now washed in distilled water for two minutes, dried in the air, and mounted in cedar oil.

Staining Method for Sections.—The nervous tissues having been hardened in Zenker's solution or sublimate alcohol are embedded in paraffin and the sections are mounted upon slides in small series.

1. The sections are treated with Lugol's solution and afterwards with a 0.5 per cent. solution of sodium hyposulphite, after which they are thoroughly washed in distilled water.

2. The sections are passed successively through 95 per cent. alcohol, absolute alcohol, chloroform, aceton, benzol, ether, absolute alcohol, 95 per cent. alcohol, and finally distilled water, in each of which reagents they remain about one hour.

3. The staining solution consists of three cubic centimeters of concentrated Giemsa solution to forty-five cubic centimeters of distilled water, in which the sections remain for twenty-four hours.

4. After brief washing in distilled water, the sections are rapidly passed in succession through aceton, aceton thirty parts plus xylol seventy parts, aceton fifty parts plus xylol fifty parts, aceton thirty parts plus xylol seventy parts, and finally pure xylol.

5. The cleared sections are mounted in cedar oil.

With these methods the minute microörganism has been demonstrated in the films in eighteen out of nineteen specimens of glycerinated human nervous tissues. It was detected regularly lying between cells and in some instances within the cytoplasm of cells, some of which were nerve cells (figures 12 and 13). The microörganism appears invariably in pairs or small aggregated groups and never in chains. The demonstration of the microorganism in sections is far more difficult, but it has been accomplished in a few instances in the interstices of the tissues and in the protoplasm of cells.

The detection of the microörganism in film preparations and in sections has been accomplished also with nervous tissues derived from monkeys inoculated at one time with the ordinary virus, at another with cultures. In this case also it is more readily brought out in film preparations than in sections. The grouping is identical with that present in the specimens of human origin.

DISCUSSION.

From the facts presented it follows that by employing a specially devised method there has been cultivated from the central nervous tissues of human beings and monkeys the subjects of epidemic poliomyelitis a peculiar minute organism that has been caused to reproduce the symptoms and lesions of experimental poliomyelitis. The microörganism consists of globoid bodies measuring from 0.15 to 0.3 of a micron in diameter, and arranged in pairs, chains, and masses, according to the conditions of growth and multiplication. The chain formation takes place in a fluid medium, the other groupings in both solid and fluid media. Within the tissues of infected human beings and animals the chains do not appear.

No statement is ventured at present as to the place among living things to which the bodies belong. It is obvious that the cultural conditions are those that apply more particularly to the bacteria.

On the other hand, the microörganism is associated with the production of an acute disease in which suppuration does not form a prominent part. No special attention at the present time has been given to the solution of the question as to whether the microorganism actually belongs to the bacteria or to the protozoa. In the manner of evolution of the symptoms, and in the appearance of the lesions, the experimental disease caused by the inoculation of the cultures resembles that produced by the virus of poliomyelitis as ordinarily employed. The central nervous organs of monkeys infected with the cultures bear preservation and glycerinization as do the infected human tissues, or the monkey tissues infected directly from human tissues. Cultures to which glycerin is directly added survive in the refrigerator at least eight days.

The microörganism passes through Berkefeld filters and the

filtrates yield upon recultivation the particular microörganism contained within the filtered culture. Moreover, Berkefeld filtrates prepared from the nervous tissues of infected human beings and monkeys yield also in culture the identical microörganism.

By employing a suitable staining method the microörganism has been detected in film preparations and sections prepared from human nervous tissues, and from the corresponding tissues of monkeys inoculated with the usual virus or with cultures or filtrates prepared from monkeys previously injected with cultures. From all the infected materials mentioned, irrespective of the manner of their origin, the microörganism has been recovered in cultures. As would be expected it is more uniformly recoverable from the original nervous tissues than from filtrates, and doubtless for the reason that in the former it exists in greater abundance.

To obtain the initial culture is difficult, and this irrespective of whether the tissues submitted to cultivation have come immediately from man or from monkeys previously inoculated with the ordinary virus or even with the cultures. Once the microörganism adapts itself to a parasitic state it is developed with greater difficulty under saprophytic conditions. Whenever the nervous tissues have been shown to be infectious, the microörganism has been recoverable, notwithstanding long preservation and glycerination. In other words, infectivity of the nervous organs and the presence of the microörganism exist together. It has indeed happened that a specimen of infected nervous tissue has at the first attempt not yielded the initial growth, although it has yielded it upon the second attempt. Persistence will usually lead to a successful cultivation, provided no technical fault is committed. An important factor in the technique of cultivation is the sample of ascitic fluid. Not all samples are suitable, and a preliminary test is necessary, using for the purpose a growing culture, in selecting samples for culture purposes. Once a suitable ascitic fluid is obtained it should be carefully husbanded in the refrigerator. Even with this precaution failure may still occur. In such an instance repetition, using the same materials but in two series, one of which is prepared for enclosure in the anaerobic jar, while the other is allowed to remain outside, may yield the desired result; or the

result may come on a second trial that appears to be an exact repetition of the first.

Only the exceptional cultures possess the degree of pathogenicity sufficient to cause specific infection, and the production of experimental poliomyelitis. A pathogenic strain may be effective at different and even remote generations, and a non-pathogenic strain may lack pathogenicity even in the second generation. This important fact indicates strongly that the pathogenic effect is not due to mere mechanical carrying over into the cultures of an invisible parasite or virus with which the cultivated microörganism is accidentally associated. If such accidental association were the cause of the experimental disease produced by the cultures in monkeys, it would display itself preferably in the first generations and without reference to the strain of the visible microörganism. On the other hand in this fluctuation of pathogenicity the cultures imitate the action of the virus as contained in human materials, namely, nervous tissue, secretions from the nasopharynx, and intestinal washings, in which the virus, either known or believed to be present, may yet fail to be demonstrated by reason of the want of infectious power for monkeys or for the particular monkey inoculated in a given instance. Moreover, it is a common experience in bacteriology to find even among the ordinary bacteria lack or rapid loss of virulence among saprophytic cultures, while virulence is not only retained, but may be increased in rapid passages from animal to animal.

In view of these considerations it would appear that an etiological relationship has been shown to exist between the cultivated microorganism and epidemic poliomyelitis as it occurs in human beings or in monkeys. There remains merely a single other possibility, already indicated, namely, that two factors are present in the cultures, the one an invisible because ultramicroscopic organism, the other the globoid bodies described. On this basis it would have to be supposed that the former but hypothetical factor is the essential agent of infection. As against this supposition it may be urged that an instance of symbiosis of this nature is not known to animal pathology. Regarding the cultivated minute but visible microörganism itself, it may be held on the basis of the data presented that it fulfills the conditions hitherto demanded for the

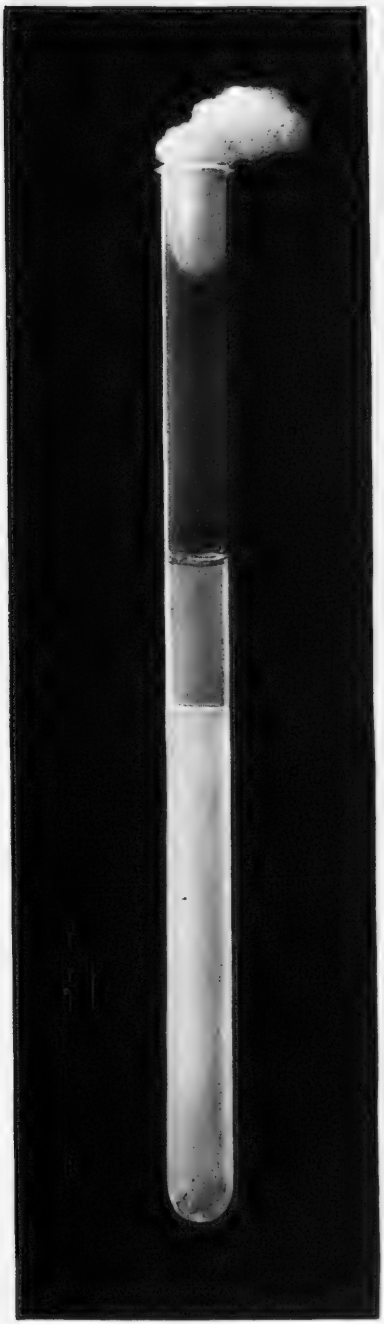
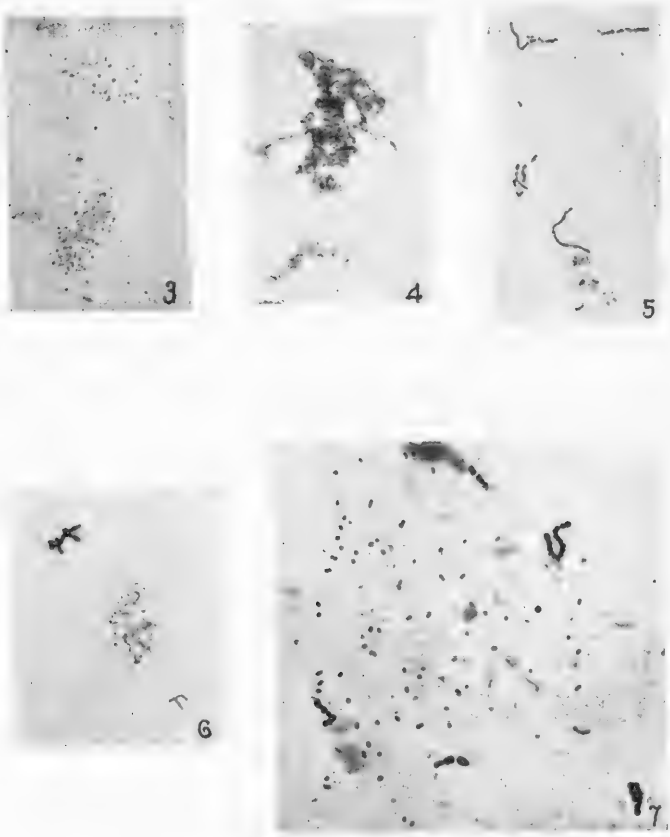


FIG. 1.

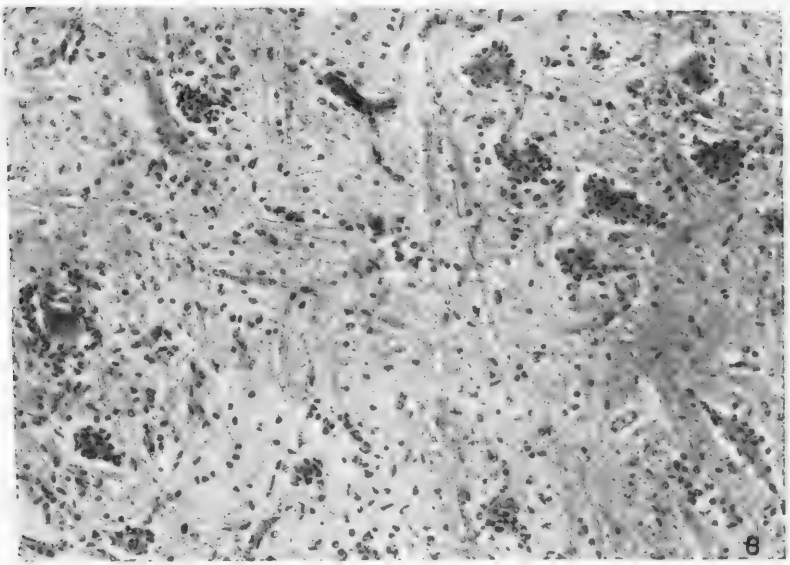
FIG. 2.

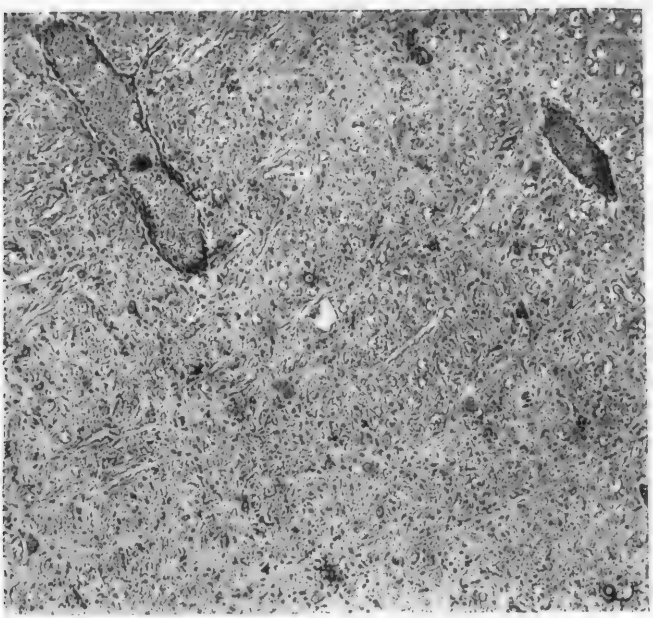
(Flexner and Noguchi: Microörganism Causing Epidemic Poliomyelitis.)

CIHM

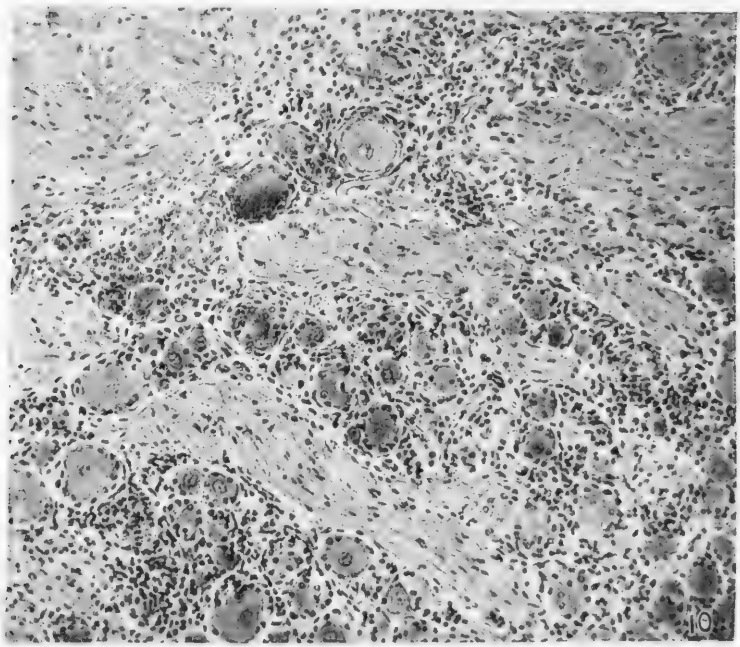


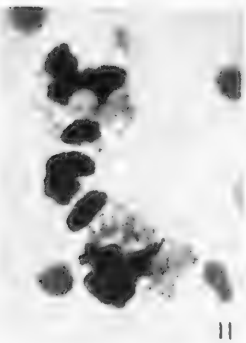
(Flexner and Noguchi: Microörganism Causing Epidemic Poliomyelitis.)

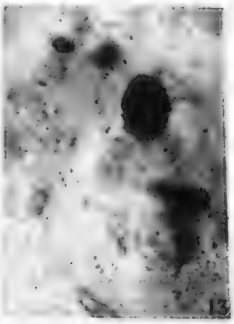




(Flexner and Noguchi: Microörganism Causing Epidemic Poliomyelitis.)







(Flexner and Noguchi: Microörganism Causing Epidemic Poliomyelitis.)

establishment of causal relation between an extraneous parasite and a specific disease. The microörganism exists in the infectious and diseased organs; it is not, as far as is known, a common saprophyte, or associated with any other pathological condition; it is capable of reproducing, on inoculation, the experimental disease in monkeys, from which animals it can be recovered in pure culture. And besides these classical requirements, the microörganism withstands preservation and glycerination as does the ordinary virus of poliomyelitis within the nervous organs. Finally, the anaerobic nature of the microörganism interposes no obstacle to its acceptance as the causative agent, since the living tissues are devoid of free oxygen and the virus of poliomyelitis has not yet been detected in the circulating blood or cerebrospinal fluid of human beings, in which the oxygen is less firmly bound; nor need it, even should the microörganism be found sometimes to survive in these fluids.

EXPLANATION OF PLATES.

Plate 38.

Fig. 1. Fluid culture showing opalescence. Natural size.

Fig. 2. Solid culture showing colonies and line of demarcation. Natural size.

Plate 39.

Fig. 3. Separate globoid bodies. × 1,000.

Fig. 4. Aggregated masses of globoid bodies. × 1,000.

Fig. 5. Chains and pairs of globoid bodies. × 1,000.

Fig. 6. Chains of globoid bodies compared with *Streptococcus pyogenes.* × 1,000.

Fig. 7. Agar fragment showing pairs of globoid bodies compared with *Streptococcus pyogenes.* × 1,000.

Plate 40.

Fig. 8. Spinal cord showing degeneration of nerve cells and invasion of leucocytes (neurophagocytosis). × 144.

Fig. 9. Perivascular and interstitial mononuclear cell infiltration of medulla. × 98.

Plate 41.

Fig. 10. Interstitial infiltration and degeneration and phagocytosis of nerve cells in intervertebral ganglion. × 160.

Fig. 11. Globoid bodies in site of inoculation taken up by leucocytes. × 1,000.

Fig. 12. Film preparation from central nervous tissue of monkey inoculated with human virus (not culture) showing pairs of globoid bodies. × 1,000.

Fig. 13. Section prepared from spinal cord of monkey inoculated with M A virus (not culture) showing intracellular pairs of globoid bodies. × 1,000.